ENVIRONMENTAL, DEVELOPMENTAL, AND BIOREGULATOR CONTROL OF FLOWERING IN MANGO (*Mangifera indica* L.)

By

ROBERTO NUÑEZ-ELISEA

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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Roberto Núñez-Elisea

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Chairman: Thomas L. Davenport, Associate Professor
Major Department: Horticultural Science

Environmental and physiological factors, as well as the effects of synthetic bioregulators, were examined in relation to flowering of mango (*Mangifera indica* L.). No changes in endogenous ethylene production were detected prior to or during floral initiation. A putative, leaf-generated floral stimulus was required during bud growth for initiation of inflorescence primordia. The stimulus persisted for about five days in stems after defoliation. Continuous cool temperatures (18°C day/10°C night) caused production of inflorescences, whereas continuous warm temperatures (30°C day/25°C night) promoted vegetative morphogenesis. Leaves became competent to stimulate flowering in response to cool temperatures at about seven

weeks of age, and at least three weeks of exposure to cool temperatures were required for floral induction. Initiation of inflorescence morphogenesis required bud growth in cool temperatures. Dormant buds are nondifferentiated, containing an arrested apical meristem and a group of preformed nodes. Bud differentiation and budbreak are continuous; primordia initiation in the preformed nodes is followed by production of new nodes by the apical meristem. A shift from warm to cool temperature regime, or vice versa, at early stages of bud differentiation, produced transitional, vegetative-to-floral or floral-to-vegetative phenotypes in a single flush of growth. Thus, temperature affects the preformed nodes and apical meristem at different times. Photoperiod was not involved in flowering. Water stress delayed vegetative flushing of shoots, but did not result in flowering, in temperatures above 20°C. With temperatures near 15°C, however, water stress caused a more rapid growth of induced buds. In cool temperatures, exogenous GA_3 repressed flowering by delaying inflorescence initiation, but it did not cause buds to grow vegetatively. Paclobutrazol and uniconazole (gibberellin-biosynthesis inhibitors) delayed vegetative flushing but did not induce flowering in warm temperatures. Both inhibitors advanced flowering in cool temperatures. In conclusion, temperatures below 20°C were conducive to floral induction and

inflorescence initiation, while temperatures above 25°C only permitted vegetative development. Bioregulators and mechanical treatments controlled the timing of bud release, whereas bud morphogenesis appeared to be regulated by the interaction, at the time of bud release and growth, of a temperature-dependent floral stimulus and floral repressor(s), presumably gibberellin(s).

CHAPTER 1
INTRODUCTION

The transition from vegetative growth to flowering in plants is of paramount importance in agriculture, horticulture, and plant breeding because flowering is the first step in sexual reproduction. The products of plant sexual reproduction account for nearly 80% of the world's food supply; however, the processes leading to flower, fruit, and seed formation remain poorly understood (Lord and Bernier, 1989).

The mango (*Mangifera indica* L.) is one of the most widely cultivated fruit crops in the world. In the tropics, only citrus and banana have greater economic value than mango (Anon., 1991). The mango producing countries include India, Mexico, Pakistan, Thailand, China (Taiwan), Indonesia, Brazil, and the Philippines (Anon., 1991). A major problem in mango cultivation concerns its inconsistent production. Yields of individual trees may fluctuate by more than 150% from one year to the next (Schaffer and Andersen, 1994). Scarce (in the tropics) or untimely (during cold weather in the subtropics) flowering are well-known limiting factors in mango cultivation (Whiley, 1993).

The mango is a polyaxial tree conforming to Scarrone's architectural model (Hallé et al., 1978), with shoot apical meristems displaying periodic vegetative extension or "flushing", and terminating by conversion to floral morphogenesis. Periodic vegetative flushing appears to be under endogenous control, as it is exhibited despite continuous presence of environmental conditions favorable for growth. Shoot apical meristems are in a state of suspended visual growth between flushing episodes, and are therefore referred to as being dormant (Lang et al., 1978). Dormancy of vegetative shoots is enhanced by cool temperatures in the subtropics, and by drought in the dry tropics (Chacko, 1986; Whiley, 1993). Flowering occurs in inflorescences produced terminally on vegetative shoots after these have experienced a period of dormancy.

Flowering is the result of complex interactions between tree condition and environmental factors. Leaves play a critical rôle in flowering, as they are the source of a putative "floral stimulus" required for induction of inflorescence morphogenesis, an event thought to take place at the time of cell division in released buds (Reece et al., 1946, 1949). Dormant buds are nondifferentiated. Dormancy release marks the start of morphogenesis, i.e., initiation and differentiation of vegetative or inflorescence primordia, a process that is continuous and not interrupted by a period of dormancy (Mustard and Lynch, 1946) as occurs

in many temperate fruit trees (Sedgley, 1990). The completion of bud differentiation is quickly followed by budbreak.

This study was conducted to gain information on the influence of environmental and internal factors in the flowering process of mango. The involvement in flowering of endogenous (ethylene) and environmental factors, and of synthetic bioregulators was investigated. Experiments were designed to distinguish whether factors under study influenced dormancy release, triggering bud growth irrespective of subsequent bud morphogenesis, or caused floral induction, that is, the programming of nondifferentiated buds for inflorescence development. Cool and warm temperature regimes, photoperiod, and water stress were tested on uniform, container-grown trees propagated by air-layering. The role of bioregulators known to promote or repress flowering of mango was examined under floral-promotive and nonpromotive environmental conditions. The translocation and longevity of the putative, leaf-generated floral stimulus was characterized by periodic girdling and defoliation of branches in field-grown trees during the flowering period. The minimum leaf age and duration of a cool temperature treatment required for floral induction were assessed in container-grown trees under controlled environmental conditions. In addition, the effects on bud morphogenesis of temperature modification during progressive stages of bud development were assessed.

CHAPTER 2
LITERATURE REVIEW

Flowering as a Developmental Process

Flowering is a progressive developmental process triggered by signals originating within the plant (Bernier, 1993). These signals may be generated in response to environmental cues, or as a result of interactions among the various plant parts independently of the environment. Most research on the regulation of flowering has been conducted in herbaceous, annual plants, which are amenable to experimentation owing to their small size, shorter life cycle, and a more predictable response to flower-promoting treatments. Photoperiod is a major environmental signal for the onset of flowering in herbaceous plants. In trees, however, temperature and water availability are the main environmental cues controlling flowering (Kozlowski, 1991).

Flowering involves a change in the developmental fate of apical meristems imposed by inductive signals (McDaniel et al., 1987). Apical meristems are determined for a vegetative developmental pattern during embryogenesis (Christianson and Warnick, 1983; Christianson, 1985; Fosket, 1994). Later in the plant's life cycle, apical meristems

gain the capacity, i.e., they become competent, to respond to inductive signals. Competence appears to be a function of both the sensitivity of the responding meristematic cells to the inductive signals and the concentration of the signals. In photoperiodic plants, exposure to favorable photoperiods causes mature leaves to produce an as yet unidentified floral-inductive stimulus. Exposure to unfavorable photoperiods, on the other hand, may result in production of floral-inhibitory signals (Bernier, 1993). The floral stimulus translocates via the phloem towards competent apical meristems, where it causes floral induction; that is, it causes an apical meristem previously determined for vegetative development, to become committed to a floral developmental pattern (Fosket, 1994). This developmental state is known as floral determination (McDaniel et al., 1987, 1992). Floral determination is generally a stable developmental state that is followed by initiation of floral morphogenesis in the presence of favorable conditions; however, it is reversible. Unfavorable photoperiods or temperatures may prevent the immediate expression of the florally determined state, and even make florally-determined apical meristems return to a vegetative developmental pattern (McDaniel, 1987, 1992).

Flowering of herbaceous plants is usually divided, for convenience of physiological investigations, into the

successive stages of floral induction, initiation, and development (Zeevart, 1976; Bernier et al., 1981a, 1981b; Thomas and Vince-Prue, 1984; Bernier, 1988; Lord and Bernier, 1989). These stages are controlled by different internal agents and may not react similarly to environmental factors (Lord and Bernier, 1989). Floral induction is followed by floral evocation, a process involving changes at the molecular and cellular levels which lead apical meristems to an irreversible commitment to floral morphogenesis (Bernier, 1988). Floral evocation and determination are distinct processes, with floral determination representing an early, or possibly the first, step of floral evocation (Kinet, 1993). Floral development involves the morphological manifestation of the floral or inflorescence structures.

Flowering in Woody Perennials

Most herbaceous and woody perennial plants use environmental cues to regulate the transition to flowering (Bernier, 1993). Synchronous flowering for successful outcrossing, as well as the completion of sexual reproduction, require favorable external conditions. Any environmental variable exhibiting seasonal change is a potential factor that may control the transition to flowering (Bernier, 1993). Although photoperiod, temperature, and water availability are the major

environmental agents controlling flowering, these may interact such that each factor can change the threshold value for the effectiveness of the others (Bernier, 1993).

Woody perennials require a longer time to achieve the ability to flower than herbaceous annuals (Jackson and Sweet, 1972). Woody perennials exhibit a prolonged juvenile period of exclusive vegetative growth before they attain the ability to initiate flowers. In mango, for example, the juvenile period can last more than six years (Singh, 1960; Chadha and Pal, 1986). Whereas many herbaceous annuals are photoperiodic with respect to floral induction, perennials generally do not rely on daylength for flowering. Flowering in woody perennials and herbaceous annuals also differs in that flowering in the latter involves the conversion of all apical meristems to the reproductive condition, whereas in perennials, some meristems can initiate flowering while other meristems continue to grow vegetatively. In other perennial polycarpic fruit trees such as *Citrus*, this behavior seems to maintain a balance between vegetative and reproductive growth, ensuring the plant's long-term subsistence (Goldschmidt and Monselise, 1970).

Owing to the above characteristics, the control of flowering in perennial, woody angiosperms is more complex than in herbaceous, annual species. Different physiological mechanisms may be involved in flowering of herbaceous and

woody perennial plants; however, it may be argued that flowering in all plants involves the same basic sequence of events (McDaniel, 1989). The separate regulation of inductive events from those concerning the initiation of morphogenesis is taken into account in flowering models proposed for the woody perennial fruit species, citrus (Davenport, 1991) and mango (Davenport, 1993).

Phenology of the Mango Tree

In attempting to study the control of flowering in mango, it is useful to consider the phenological behavior of the tree throughout the year. The vegetative growth and flowering patterns of mango trees differ in areas with marked changes in environmental conditions (subtropics and dry tropics) and areas with mostly uniform environmental conditions (wet tropics). An empirical model describing the phenological behavior of mango trees in sub-tropical conditions has been proposed by Cull (1987, 1991). Trees growing in these conditions exhibit distinct phenological phases through the year in response to the marked seasonal changes. Tree canopies are either dormant, in active vegetative growth, in bloom, or fruiting, with little overlapping of these developmental stages. Vegetative growth commences after fruit is harvested in the warm summer months and extends into the fall, and is believed to be accompanied by a flush of root growth (Cull, 1987). Soon

afterwards, trees cease vegetative growth for a period of up to three months. Inflorescence buds are initiated during the cool fall-winter months in response to low-temperature, and flowering follows soon afterwards in the late winter-early spring. This phenological cycle is repeated every year. The occurrence of a cool winter period in the sub-tropics promotes temporal alternation of vegetative and reproductive phases, contributing to a dependable flowering and fruit production (Cull, 1987; Verheij, 1986). However, the sporadic occurrence of frosts or freezes during the winter in the subtropics, coinciding with the flowering of trees, may reduce fruit yield obtained during the summer.

Episodes of drought rather than low temperature intensify vegetative dormancy and determine the flowering pattern of mango trees in the tropics (Chacko, 1986). The frequency, duration, and intensity of wet and dry periods can vary greatly among tropical environments, resulting in highly variable phenological patterns. For example, mango trees growing in wet tropical conditions simultaneously display different phenological stages in different sectors of the canopy. During mild winters in these areas, some branches may be flushing vegetatively, while other branches may be dormant, flowering, or carrying fruit (Cull, 1987). Such erratic growth pattern is a major cause of low productivity of mango under wet tropical conditions, as tree

resources are diverted to support both vegetative and reproductive development at the same time.

Mango trees growing in the dry tropics, as those growing in the subtropics, display a defined temporal separation of the vegetative and flowering stages. Tropical areas with a 3- to 5-month dry season are considered ideal for mango cultivation due to alternation of the vegetative and reproductive phases and the absence of damaging frosts during the flowering and early fruit set period.

Shoot Development from Vegetative Extension to Floral Initiation

Shoots of some angiosperms, including mango, exhibit an initial phase of vegetative development and terminate by flowering (Hallé et al., 1978; Steves and Sussex, 1989; Chasan and Walbot, 1993). Most shoots of mango are produced immediately after fruit is harvested, and continue elongating until terminal flowering ensues. A flowering shoot consists of both a vegetative portion that includes one or more flushes (segments) of vegetative growth, and the apical inflorescence.

Shoot growth during the vegetative phase is monopodial and characterized by periodic terminal "flushing" (Valmayor, 1968; Chacko, 1986; Hallé et al., 1978; Verheij, 1986), rather than continuous growth. Vegetative flushing of

shoots is also referred to as "extension" or "elongation" (Singh et al., 1960; Hallé et al., 1978). Flushing episodes are interceded by a period of dormancy lasting from a few weeks to several months (Nakasone et al., 1955; Singh, 1960; Beal and Newman, 1986; Issarakraisila and Considine, 1991). Each new vegetative flush is produced after leaves of the most recently formed flush have expanded (Chacko, 1986; Whiley, 1993), which occurs about 3 to 4 weeks after budbreak. The vegetative portion produced prior to flowering may comprise one or more flushes of extension growth.

Nearly all inflorescences in a mango tree arise from apical buds of vegetative shoots produced within the previous year. Flowering shoots, which are located on the periphery of the tree canopy, are the main source of new vegetative shoots for the current season. It is from their distal axillary buds (those located immediately below the insertion of the apical inflorescence), that the new vegetative shoots arise after the inflorescence is detached. Inflorescences can also arise directly from axillary buds on a vegetative shoot, or from lateral buds on a branch or the main trunk of a tree (for example, as a result of pruning during the flowering period), without the initial formation of a vegetative portion.

An important feature of mango is that dormant buds (whether apical, axillary, or lateral) lack differentiated vegetative or floral primordia, containing only an apical meristem and a few preformed nodes in an arrested state of development. Vegetative or inflorescence bud differentiation ensues immediately upon dormancy release and is a continuous process not interrupted by a period of dormancy (Mustard and Lynch, 1946). Bud differentiation is quickly followed by bud break and expansion of the new flush of growth, whether vegetative or generative. In contrast to mango, reproductive buds of temperate fruit trees such as apple initiate floral primordia in the summer or autumn soon after the current season's flowering and then lie dormant over winter. Anthesis is reached the following spring, about 9 to 11 months after buds initiated growth (Buban and Faust, 1982; Sedgley, 1990). Floral development in mango, from the onset of floral initiation to anthesis, is generally completed within two months (Singh, 1960; Scholefield et al., 1986).

Involvement of a Floral Stimulus in Mango Flowering

In common with floral induction in many herbaceous plants, leaves play an essential morphogenic role in flowering of mango. Reece et al. (1946, 1949) in Florida provided the first evidence that a floral stimulus produced by mature leaves was necessary for initiation of

inflorescence primordia in mango buds. Treatments combining defoliation, bark ringing, and decapitation of mature vegetative shoots were imposed during the early flowering period of 'Haden' mango trees. The role of leaves in flowering was examined by simultaneously defoliating and decapitating mature shoots. Shoots were either ringed or not ringed to observe whether the released axillary buds differentiated as new vegetative shoots or inflorescences. In ringed shoots, axillary buds only produced vegetative shoots, whereas in nonringed shoots, axillary buds produced inflorescences. Defoliation prior to the start of bud growth prevented floral initiation and resulted in vegetative growth, whereas defoliation after bud growth had commenced resulted predominantly in floral initiation. These contrasting responses indicated that a leaf-generated signal was translocated via the phloem to instruct actively dividing meristematic cells to initiate floral primordia. The results thus showed that cell division was triggered by shoot decapitation, which released axillary buds from dormancy, whereas induction of floral morphogenesis was mediated by the floral stimulus generated in the leaves (Reece et al., 1946, 1949). The inductive capacity of mango leaves appears to be age-dependent since flowering occurs only on shoots having mature, fully expanded leaves.

Following the significant observations of Reece et al. (1946, 1949), Singh (1959) achieved graft-transmission of the floral stimulus from mature to juvenile mango trees. Floral initiation in the juvenile receptor indicated that juvenile meristems were florally competent, and suggested that juvenility in mango was due to the incompetence of leaves to produce floral stimulus. Graft-transmission of floral stimulus from adult to juvenile receptor trees was later attempted, unsuccessfully, by Kulkarni (1986). In later experiments, however, Kulkarni (1988b) achieved transmission of the floral stimulus from adult, off-season flowering trees to adult trees which were not in their flowering period.

Initiation of Inflorescence Morphogenesis

As noted earlier, dormant buds show no histological evidence of vegetative or reproductive activity. They contain only an apical meristem surrounded by a few nodes consisting of an inner scale and axillary meristem (Lanuza, 1937; Mustard and Lynch, 1946; Ravishankar et al., 1979; Scholefield et al., 1986; Sen and Mallik, 1941; Singh, 1958).

The first indication of reproductive development in mango buds is initiation of inflorescence primordia in the axillary meristems of preformed nodes (Sen and Mallik, 1941; Mustard and Lynch, 1946). Each inflorescence primordium

develops into a secondary axis of the panicle and bears numerous individual flowers.

Environmental Influences upon Vegetative Growth

Dormancy and its release appears to be under endogenous control (Hallé et al., 1978). Vegetative shoot dormancy is promoted by environmental conditions such as a period of drought in the seasonal tropics or low night temperatures in the subtropics (Ou, 1982; Chacko, 1986; Verheij, 1986; Whiley, 1993). The time interval between episodes of vegetative flushing is shortened under warm temperatures about 20°C or higher (Shu and Sheen, 1987; Whiley et al., 1989; Ravishankar et al., 1979), abundant water availability, and high nitrogen level (Cull, 1987).

Temperature

Controlled-environment studies with several mango cultivars have shown that the mean temperature for cessation of shoot extension is 15°C, and that the frequency of shoot extension increases with increasing temperatures (Whiley et al., 1989). For a given temperature, the duration of the dormant period between extension episodes varies greatly among cultivars. For example, a mean temperature of 27.5°C caused shoots of 'Irwin' mango to remain dormant for 45 days, while shoots of 'Kensington' remained dormant for only 5 days (Whiley et al., 1989). Limited shoot extension is a

desirable trait because it reduces canopy size and facilitates tree management.

Water status

Plant water status also affects vegetative growth of mango, as shoots of trees under water deficit cease to flush due to lack of turgor necessary for cell expansion and growth of the apical bud. For example, leaf water potentials of mature 'Nam Dok Mai' trees exposed to 5 weeks of drought fell to -0.8 MPa and no vegetative flushing occurred during this period (Whiley, 1993). Larson et al. (1989) reported that leaf water potentials of -0.2 to -0.5 MPa did not restrict vegetative flushing of field-grown 'Tommy Atkins' mango trees in South Florida.

Photoperiod

No studies seem to exist on the influence of daylength on mango shoot extension. Seasonal daylengths within the range in which the mango is cultivated throughout the tropics and subtropics (about 10.5 h to 13.5 h) do not appear to influence the frequency of vegetative flushing.

Environmental Influences upon Floral Induction and Floral Initiation

Temperature

Temperature has been demonstrated to exert a critical influence in flowering. Cool night temperatures between 8°C and 15°C in combination with day temperatures below 20°C typically induce floral initiation (Ou, 1982; Shu and Sheen,

1987; Whiley et al., 1989; Wolstenholme and Mullins, 1982). Buds of intact trees subjected to cool temperatures (15°C day/10°C night) required at least 120 days to initiate and complete inflorescence differentiation, and to attain subsequent budbreak (Whiley et al., 1989). The time of exposure to low temperatures required to achieve floral induction has not been determined.

Production of floral buds is most erratic under continuous warm temperatures (20°C or more), and high soil and atmospheric moisture, because these conditions are conducive to vegetative flushing and do not allow for the sustained period of dormancy that seems to be required for floral initiation (Wolstenholme and Mullins, 1982; Chacko, 1986; Whiley, 1993).

Water Stress

In the absence of cool temperatures below 15°C, mango trees in the tropics seem to initiate inflorescences in response to plant water deficit after a 2- to 3-month period of drought (Chacko, 1986). Few studies have been conducted to examine the effect of water deficit on mango flowering. Floral initiation was not achieved in containerized mango trees subjected to eight weeks of water deficit as determined by soil moisture content (Wolstenholme and Hofmeyr, 1985).

It is well known that floral initiation of mango trees growing in the seasonal tropics occurs after a period of drought. In the subtropics, floral initiation ensues during cool weather in the fall an d winter, apparently irrespective of plant water status. Mango trees in the tropics are believed to rely less on cool temperature for floral induction than trees growing in the subtropics (Cull, 1987; Singh, 1960; Singh, 1978; van der Meulen et al., 1971). Although it has been assumed that water stress causes floral induction of mango (Singh, 1960; Grierson et al., 1982; Wolstenholme and Mullins, 1982), this notion has not been demonstrated experimentally. More research is therefore needed to decipher the role of plant water deficit in mango flowering.

Daylength

A role for photoperiod in mango flowering has not been demonstrated, and there is no clear indication that this environmental factor plays a significant influence in the transition to reproductive development. Mango trees in the subtropics, around 25° N or S latitudes, initiate inflorescence development and bloom during the shortest days of the year (10.5 to 11.0 hours daylength), which occur during the fall and winter seasons. Yet, flowering typically occurs earlier the closer the location to the equator (Chacko, 1986), where daily photoperiods approximate

12 hours year-round. It seems more likely that floral initiation near the equator occurs in response to plant water deficit during periods of drought, and that warm temperatures, available at all times, expedite inflorescence development.

Cool temperature in the subtropics or drought in the tropics appear more critical than daylength for floral initiation in mango. To assess the influence of photoperiod in mango flowering, it is necessary to expose trees to a range of daylengths under both inductive and noninductive temperature regimes.

Manipulation of Flowering

Correlative Modifications

Shoot or branch structure can be modified by procedures such as defoliation, ringing, tipping (shoot decapitation), or shoot deblossoming (removal of the apical inflorescence). These procedures influence floral initiation by altering (1) the time of bud dormancy release, (2) production of floral stimulus (defoliation), and/or (3) transport of floral stimulus to bud meristems (ringing). For example, deblossoming stimulates growth of dormant axillary buds, and these will differentiate as inflorescences provided bud differentiation occurs under conditions conducive to floral morphogenesis (Reece et al., 1946, 1949). However, when deblossomed shoots are simultaneously defoliated and ringed,

the released axillary buds will only produce new axillary vegetative shoots, even if bud differentiation occurs under floral-inductive temperatures, because ringing prevents translocation towards the activated buds of floral stimulus generated in distant leaves (Reece et al., 1946, 1949).

Synthetic Bioregulators

Chemical bioregulators have been used to promote or discourage flowering. The main approaches include (1) forcing dormant buds to undergo growth and differentiation during the period conducive to floral initiation; (2) repressing vegetative flushing of shoots to increase the probability of floral initiation upon arrival of floral-promotive conditions; (3) preventing or delaying floral initiation during the floral-inductive period, and (4) causing abscission of apical panicles to promote a delayed axillary bloom.

Forcing bud release. Compounds that have proven effective in breaking bud dormancy include potassium nitrate (KNO_3; Bondad and Linsangan, 1979; Mosqueda-Vázquez and de los Santos de la Rosa, 1981; Núñez-Elisea, 1985, 1986), ammonium nitrate (NH_4NO_3; Núñez-Elisea, 1988; Núñez-Elisea and Caldeira, 1988), and ethephon (2-chloroethyl phosphonic acid; Chacko et al., 1971, 1972 1974a, 1974b; Dutcher, 1972; Chen, 1985; Choudhuri and Rudra, 1971; Maity et al., 1972; Núñez-Elisea et al., 1979; Pandey et al., 1973; Sen et al.,

1973). These chemicals are applied as foliar sprays a few weeks prior to the normal start of flowering.

Chemical bud forcing is most effective in areas around the equator. The response to chemical bud forcing diminishes at latitudes beyond about 22° N or S, a fact that has not been clarified but may involve the decline of night temperatures from about 20°C around the equator to less than 10°C between 22° and 35° N or S latitude. Extended periods of lower temperatures possibly induce a more profound shoot dormancy prior to floral initiation, rendering the potentially inflorescence buds insensitive to conventional chemical stimulation.

The role of KNO_3 in mango flowering has been proposed to be inductive (Barba, 1974), that is, that KNO_3 programs nondifferentiated buds for inflorescence development when they would otherwise develop vegetatively. One would expect, however, that if KNO_3 caused floral induction, its use would stimulate floral initiation at any time of the year. In Mexico, 'Haden' and 'Manila' trees typically continue to flush vegetatively rather than produce bloom when KNO_3 or NH_4NO_3 are sprayed between June and September, the warm, rainy season conducive to vegetative growth (R. Núñez-Elisea, unpublished results). Inflorescence buds are formed only when trees are sprayed after mid-October, and the proportion of flowering shoots increases as sprays are

applied closer to the normal flowering period in January-February. These results indicate that KNO_3 and NH_4NO_3 release bud dormancy, triggering bud growth and differentiation, but do not determine the course of bud differentiation. Upon release from dormancy as a result of KNO_3 and NH_4NO_3 treatment, it is likely the floral stimulus produced in mature leaves, not nitrate, that induces meristems to initiate inflorescence primordia. Kulkarni (1988b) shares the view that the floral stimulus is already present in shoots at the time that buds are stimulated to grow in response to KNO_3, and further suggests that KNO_3 may, in addition to releasing bud dormancy, sensitize buds to the floral stimulus.

The stimulation of mango flowering with ethephon (Chacko et al., 1972, 1974a; Sen et al., 1973), which is metabolized within plant tissues and releases ethylene as a by-product, led to the hypothesis that endogenous ethylene causes floral induction. Saidha et al. (1983), working with the Indian cultivars, Mulgoa, Neelum, and Bangalora, reported a gradual increase in internal leaf ethylene production from June to November, as the season of floral initiation approached. At the time of inflorescence budbreak in December, ethylene production in leaves of flowering shoots was up to five-fold that of leaves from dormant vegetative shoots. Ethylene production during

development of vegetative buds was not measured in that study. Such information appears necessary to specify an involvement of ethylene in the transition of shoots to the flowering stage. Whether changes in ethylene production observed in the study by Saidha (1983) occur under Florida conditions and in cultivars originated in Florida, such as 'Tommy Atkins', is not known.

Inhibition of vegetative flushing. Application of growth retardants several months before the flowering period represses production of vegetative flushes and results in earlier flowering compared to nontreated trees. These compounds include ethephon (Chacko et al., 1971, 1974a; Sen et al., 1973), chlormequat (Suryanarayana and Rao, 1978), daminozide (Suryanarayana and Rao, 1978), and more recently the gibberellin-synthesis inhibitors, paclobutrazol (Kulkarni, 1988a; Goguey, 1990; Burondkar, 1991; Charnvichit et al., 1991; Hillier, 1991; Rowley, 1990; Tongumpai et al., 1991; Voon et al., 1991), and uniconazole (Rukayah, 1990). Gibberellins have been demonstrated to inhibit initiation of inflorescence development in mango (Kachru et al., 1971). Paclobutrazol and uniconazole also suppress vegetative flushing and reduce internode length. Paclobutrazol modifies the flowering habit of otherwise intact trees, causing floral initiation in axillary and lateral buds which normally remain dormant and nondifferentiated (Burondkar and

Gunjate, 1993; Kulkarni, 1988). Experiments conducted under controlled, floral-promotive and nonfloral-promotive environmental conditions are necessary to clarify whether paclobutrazol and other bioregulators influence flowering independently of environmental conditions.

Inhibition or delay of apical flowering. In many woody angiosperms, initiation of floral primordia is repressed by applications of gibberellic acid (GA_3; Pharis and King, 1985). GA_3 antagonizes flowering of several temperate and tropical fruit crops, including mango (Kachru et al., 1971; Rawash et al., 1983; Tomer, 1984; Andrews and Le Fook, 1985; Galán-Saúco, 1990). In mango, GA_3 foliar sprays close to the flowering period greatly reduced flowering of 'Haden', 'Keitt', 'Palmer', and 'Tommy Atkins' (Tomer, 1984), as well as of 'Dashehari' trees (Kachru et al., 1971).

Bloom delay with GA_3 can be useful in areas where recurring temperatures below 5°C stimulate production of apical inflorescences, but their continued presence hampers pollination, fertilization, and early fruit development (Young and Sauls, 1979; Wolstenholme and Mullins, 1982; Galán-Saúco, 1991). Low temperatures cause production of seedless, underdeveloped fruit which lack commercial value (Young and Sauls, 1979). GA_3 is used commercially in South Africa (S. A. Oosthuyse, personal communication) and Israel (Tomer, 1974) to prolong shoot dormancy and prevent

flowering of young trees during the winter, thereby canalizing tree resources towards formation of canopy structure.

Although GA_3 inhibits flowering in mango, it is not clear whether it can cause buds to develop vegetatively under floral-inductive conditions, when they would be expected to undergo inflorescence morphogenesis. In 'Dashehari' mango, GA_3 applied to dormant apical buds near the time of floral initiation was reported to cause initiation of vegetative primordia (Kachru et al., 1971). Vegetative primordia in GA_3-treated buds, however, were initiated several weeks later than floral primordia in nontreated, control buds, suggesting that vegetative primordia may have been initiated after floral-inductive conditions receded, and not in direct response to GA_3. An indirect role of GA_3 would seem supported by the fact that GA_3 results in delayed floral initiation only when buds undergo growth and differentiation during cool, floral-promotive weather conditions (Galán-Saúco, 1990). Once these conditions recede (around mid-spring), activated buds will develop vegetatively rather than form inflorescences.

<u>Delay of axillary flowering by deblossoming.</u> Late blooms can be promoted with the use of abscission agents such as the ethylene-releasing compounds, ethephon (Chadha et al., Galán-Saúco et al., 1993) and cycloheximide (Pal and

Chadha, 1982; Shu, 1993). Sprays of these compounds cause abscission of apical panicles, thereby releasing dormant axillary buds. The released axillary buds will produce inflorescences when undergoing growth in cool, floral-inductive weather, but they will develop as vegetative shoots if growth occurs during warm temperatures.

Requirements for an Experimental Flowering System in Mango

Studies on the control of flowering in woody perennial fruit trees, including mango, have been limited by the lack of experimental systems that allow control over floral initiation (Jackson and Sweet, 1972; Wareing, 1981; Chacko, 1984; Hackett, 1985). Floral initiation of perennial trees can occur under alternative environmental pathways (Bernier, 1988; Evans, 1969; Sedgley, 1990). In mango, for example, floral initiation occurs by exposure of trees to temperatures of 15°C or less (Ou, 1982; Shu and Sheen, 1987; Whiley et al., 1989; Wolstenholme and Hofmeyr, 1985) or after a drought period of 2 to 3 months in field-grown trees (Chacko, 1984; Singh, 1960; Verheij, 1986) with temperatures above 15°C. Cool temperatures or water stress are reported to induce flowering of containerized 'Tahiti' lime trees (Southwick and Davenport, 1986).

Mango trees growing under natural conditions attain large size and display sporadic flushes of vegetative or floral growth (depending on the season) in different

sections of the canopy. Asynchronous canopy growth may also be influenced by heterogeneous rootstocks and differences in soil moisture or nutrient availability within the root zone. Experimental flowering systems should ideally minimize both plant and environmental variability, and give consistent and predictable flowering responses. The identification of environmental and developmental factors promoting and preventing floral initiation may be achieved by using small, nonjuvenile trees in controlled environmental conditions.

CHAPTER 3
PRODUCTION OF HOMOGENEOUS PLANT MATERIAL FOR FLOWERING STUDIES

Asexual propagation by air-layering allows rapid production of mature trees of many tropical fruit species including mango (Chaudri, 1976). Air-layering of mango has been demonstrated in Indian cultivars using NAA and other synthetic auxins (Chaudri, 1976; Rao, 1967; Singh, 1960; Valmayor, 1968). The technique ensures genetic uniformity of experimental plant material and eliminates possible rootstock influences on the response to environmental conditions. The degree of success in air-layering varied with cultivar, branch age and season of treatment. Indian cultivars which rooted well included 'Dashehari', 'Machhali', 'Gulab Khas', 'Bombay', and 'Himsagar'. In contrast, 'Langra' developed few roots (Basu et al., 1969; Singh and Teaotia, 1961). Singh (1954) mentioned that shoots less than two years old were most adequate for air-layering.

Shoots treated with NAA at 0.5% or 1.0% NAA produced 100% rooted air-layers and all rooted air-layers of the latter treatment survived establishment in the field

(Srivastava, 1960). In the same study, IBA (indolebutyric acid) at the same rates also showed acceptable root promotion. Singh (1954) obtained 100% rooting in air-layers of 'Dashehari' using 1.0 and 2.0% NAA in lanolin, but the same treatments produced only 50 and 60% rooting in 'Langra'. In this experiment the efficacy of NAA for inducing adventitious root formation in air-layers of 'Tommy Atkins' mango was examined. The objective was to find a reliable method for producing small, developmentally uniform trees capable of initiating flowering for research on flowering under controlled growth and environmental conditions.

Materials and Methods

Air-layers were made in fifteen 12-yr-old 'Tommy Atkins' mango trees during the last week of July, 1988. The treatments tested were: 0, 0.1, 0.5, 1.0, and 2.0% NAA in lanolin. Three branches were used per each of five treatments per tree (15 air-layers per tree). Two- to three-year-old branches measuring 1.5 to 2.0 cm in diameter were selected. Selected branches had brown, corky bark, as opposed to the green, smooth bark of younger branches. A 5-cm girdle of bark was removed from each branch.

To achieve each NAA rate, the corresponding amount of pure NAA was dissolved in 1 ml acetone and the solution blended into 10 g warm, liquified anhydrous lanolin. The

paste was mechanically stirred until the acetone had evaporated, and then stored in 10-ml syringes. Approximately 0.3 cm^3 of lanolin paste was applied to the upper rim of each girdle and rubbed in a band about 3 cm wide. Control branches were girdled and treated with lanolin only. Girdles of all air-layers were covered with a handful of moist sphagnum moss and wrapped with extra duty aluminum foil. The number of leaves and shoots per branch, as well as branch diameter 5 cm above the girdle were recorded to assess their possible influence on rooting.

Root development was inspected periodically. Twelve weeks after treatment, virtually all air-layers with 2.0% NAA had fully-developed root systems (stage 5 of Table 1). At this time, all layers were detached from mother trees and root development classified into the stages described in Table 3-1.

To evaluate survival in containers, all air-layers with roots in stages 4 and 5 were defoliated by about 20% to reduce transpiration, and potted in 4-liter containers by the end of October. The soil mix consisted of peat moss, sand, and perlite (1:1:1 by volume) plus 60 g of Osmocote slow-release fertilizer. The potted air-layers were placed under shade and drip-irrigated for 30 min during the morning on a daily basis.

Table 3-1. Root developmental stages of 'Tommy Atkins' mango air-layers.

Stage	Morphological characteristics
0	No basal swelling, no visible root primordia.
1	Basal swelling, no visible root primordia.
2	Root initials up to 1 cm long.
3	Primary roots 1 to 5 cm long.
4	Primary roots > 5 cm long, few lateral rootlets.
5	Primary roots > 5 cm long, many thin, lateral rootlets about 5 mm long.

Results and Discussion

Effect of NAA Dose on Adventitious Rooting

The dose of exogenous auxin influenced the degree of adventitious rooting in 'Tommy Atkins' mango air-layers. The average root stage (sum of individual ratings divided by 45) increased as the rate of NAA increased (Fig. 3-1).

The proportion of air-layers with roots in each developmental stage within treatments is shown in Fig. 3-2. Air-layers treated with 0.1% NAA produced mainly stage-2 and stage-3 root systems (about 30% of each), with about 20% of air-layers producing stage-4 and stage-5 roots. Nearly all air-layers (93%) treated with 2.0% NAA produced stage-5 roots, while only 55% of air layers treated with either 1.0% or 0.5% NAA produced stage-5 roots. NAA at 0.1% and 0.5% produced stage-5 roots in only 13% and 4% of air-layers, respectively. Maximum root development in control air-layers was stage 3, with only 7% of air-layers reaching this category.

A previous report indicated that the best rooting of 'Deshehari' mango was obtained in branches less than 2 years old (Singh, 1954). However, this experiment showed that 2- to 3-yr-old branches of mature 'Tommy Atkins' mango trees can produce excellent rooting with the aid of 2.0% NAA in lanolin.

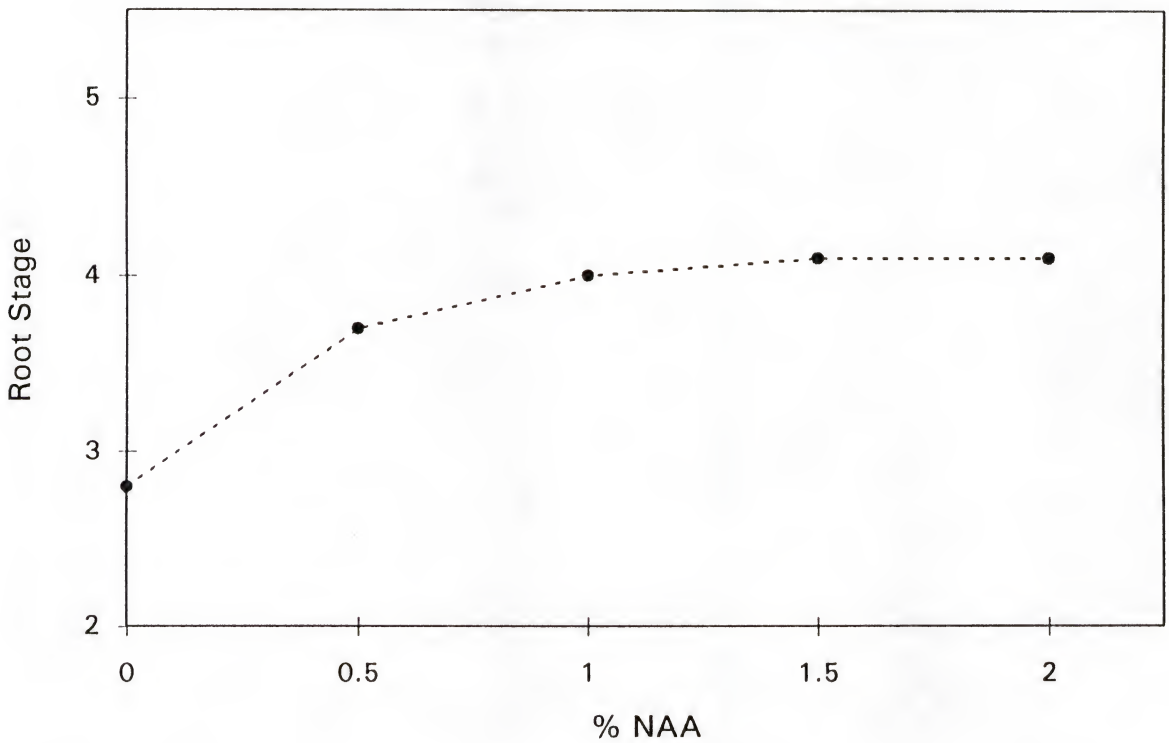

Figure 3-1. Rooting of 'Tommy Atkins' mango air-layers in response to NAA concentration. The effect of NAA on root developmental stage is defined by the equation: $Y = 4.065 + 0.339 \text{ Ln } X$ ($R^2 = 0.54$). Data points were estimated by SAS nonlinear regression.

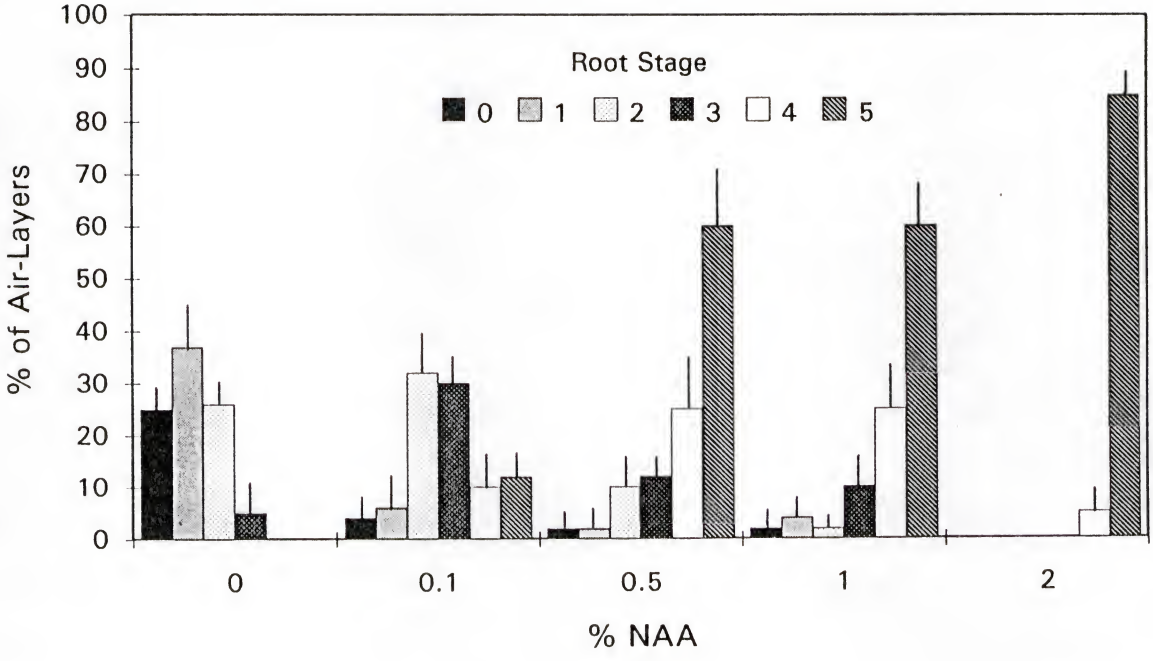

Figure 3-2. Distribution of root stages within NAA treatments applied to 'Tommy Atkins' air-layers. (See Table 3-1 for description of root developmental stages).

Survival rate of air-layers after potting

Survival rate after potting was greater for air-layers with stage-5 than stage-4 roots (96% vs 65%). The enhanced production of thin, lateral rootlets in stage-5 root systems likely increased survival rates by allowing a more efficient uptake of water and nutrients (Singh, 1978), and perhaps also by supplying the aerial system with endogenous growth substances.

Growth of surviving air-layers was not influenced by root stage at the time of potting. Examination of root development 30 days after potting revealed that both initially stage-4 and stage-5 root systems contained abundant lateral rootlets.

Shoots of all air-layers remained dormant for 2 to 3 months after potting, and flowered in January-February of 1989, when mother trees in the field were also in bloom. Flowering of mature 'Tommy Atkins' mango trees in south Florida typically occurs at this time of year (Young and Sauls, 1979), when cool temperatures set in . Thus, the flowering behavior of 'Tommy Atkins' mango trees produced by air-layering was similar to that observed in field-grown trees.

Results of this study demonstrated that 'Tommy Atkins' mango can be readily propagated by air-layering with the aid of NAA for induction of rooting, and that experimental trees

obtained by this procedure are capable of flowering shortly after potting. Therefore, air-layered trees were selected as standard experimental plant material for studying the environmental regulation of flowering under controlled environmental conditions, and the effects of various synthetic bioregulators on grwoth and flowering.

CHAPTER 4
ROLE OF ENDOGENOUS ETHYLENE IN FLOWERING

The use of smoke ("smudging") to stimulate flowering of mango (Gonzales, 1923) has long been thought to involve ethylene generated by burning material (Dutcher, 1972; Bueno and Valmayor, 1974). The subsequent use of ethephon to promote mango flowering led to the hypothesis that endogenous ethylene causes floral induction (Barba, 1974). Ethephon is an effective floral promoter of mango trees growing in the tropics (Chacko et al., 1971, 1972 1974a, 1974b; Choudhuri and Rudra, 1971; Dutcher, 1972; Maity et al., 1972; Pandey et al., 1973; Sen et al., 1973; Nuñez-Elisea et al., 1979; Chen, 1985). Symptoms that may indicate a participation of ethylene in mango flowering include the extrusion of latex in apical buds and epinasty of leaves near the apex at the time of floral budbreak.

Stimulation of early flowering in mango with KNO_3 under tropical conditions (Bondad and Linsangan, 1979; Mosqueda-Vázquez and de los Santos de la Rosa, 1981; Núñez-Elisea, 1985, 1986) has also been speculated to involve increased ethylene production (Barba, 1974). Mosqueda-Vázquez and Avila-Reséndiz (1985) reported that the efficacy of KNO_3 was

negated by $CoCl_2$ and $AgNO_3$, compounds which inhibit the synthesis and action of ethylene, respectively, when sprayed 1 to 4 hr after KNO_3. They also refer to the work of Thuck-Thye (1978), who linked the efficacy of KNO_3 to ethylene production. Saidha et al. (1983) reported an increase in internal leaf ethylene production as the season of floral initiation approached. Ethylene production by flowering shoots was up to five-fold that of vegetative ones.

The present experiments were conducted to examine ethylene production by different plant parts of 'Tommy Atkins' mango trees during floral inductive and noninductive conditions.

Materials and Methods

Plant Material

Leaves, stems, and buds collected for ethylene measurements were collected from 8-yr-old 'Tommy Atkins' mango trees growing in a calcareous soil under subtropical conditions in Homestead, Florida. Chemical spray treatments were also applied on such trees.

Ethylene Measurement

For all experiments, container ethylene concentration was determined from 1-ml gas samples injected onto a Varian 3700 gas chromatograph equipped with a 2 m x 3 mm alumina column maintained at 100°C and a N_2 flow rate of 30 $ml \cdot hr^{-1}$.

The sensitivity level for ethylene in this system was 5 $nl \cdot l^{-1}$.

Ethylene Production by Buds, Leaves, and Stems at the Start of the Flowering Period

Ethylene production was measured in shoots with dormant or breaking apical buds in December, 1988. Excised apical buds, leaves, and 10-cm decapitated, defoliated stem segments were separately assayed for ethylene synthesis. Ethylene measurements were made by enclosing the buds, leaves, or stem segments for 1 hr into 30, 50, or 250-ml air-tight glass containers, depending upon sample size.

Ethylene Production after KNO_3 Sprays Applied During the Floral-inductive Period

Sprays of 8% KNO_3 or water were applied on individual scaffold branches during the floral-inductive period. Ethylene production by excised leaves collected at various time intervals after spraying and the flowering behavior of the branches in the field were compared.

Ethylene Production During the Nonfloral-inductive Period in Response to Chemical Sprays (Single Leaf Sampling)

Spray treatments of KNO_3 (2% or 8%) or ethephon (125, 500, and 2000 $mg \cdot l^{-1}$) were applied in June, 1989, to scaffold branches of field-grown trees. Control branches were sprayed with water. Leaf samples were collected from each treatment immediately after spraying and ethylene measured at various time intervals during a 98-hr time course. Containers were left open to the laboratory atmosphere

between measurements. Leaf dehydration was avoided by introducing moist paper tissue in the containers between ethylene sampling times.

Ethylene Production During the Nonfloral-inductive Period in Response to Chemical Sprays (Periodic Leaf Sampling)

In a final experiment conducted with field-grown trees in June, 1989, ethylene production was measured for 87 hr in leaves collected at various time intervals from branches sprayed with 2000 $mg \cdot l^{-1}$ ethephon, 8% KNO_3, or water.

Results

Ethylene Production by Buds, Leaves, and Stems at the Start of the Flowering Period

Bud, leaf, and stem samples taken from mature shoots of orchard trees during the cool, floral-inductive period showed minimal ethylene production rates typical of other mango tissues (Núñez-Elisea and Davenport, 1983, 1986; Table 4-1). All three organs demonstrated no change in ethylene production over basal levels during bud development and bud break.

Ethylene Production after KNO_3 Sprays Applied During the Floral-inductive Period

Ethylene production by leaves collected at various time intervals from branches sprayed with 8% KNO_3 during the inductive period was not significantly different from that of water-sprayed controls (Figure 4-1). In the field, KNO_3 sprays had no effect on flowering, as both treated and

Table 4-1. Ethylene production by apical buds, leaves and shoot stems of 'Tommy Atkins' mango during floral inductive conditions (December, 1988). Each datum is the mean of five samples ± SE. Dormant buds were nondifferentiated.

	Ethylene production ($nl \cdot g^{-1} \cdot hr^{-1}$)		
Bud stage	Shoot apex	Leaves	Stems
Dormant	1.84 ± 0.09	1.00 ± 0.05	0.90 ± 0.06
Breaking infl. bud	2.06 ± 0.12	1.22 ± 0.08	0.68 ± 0.04

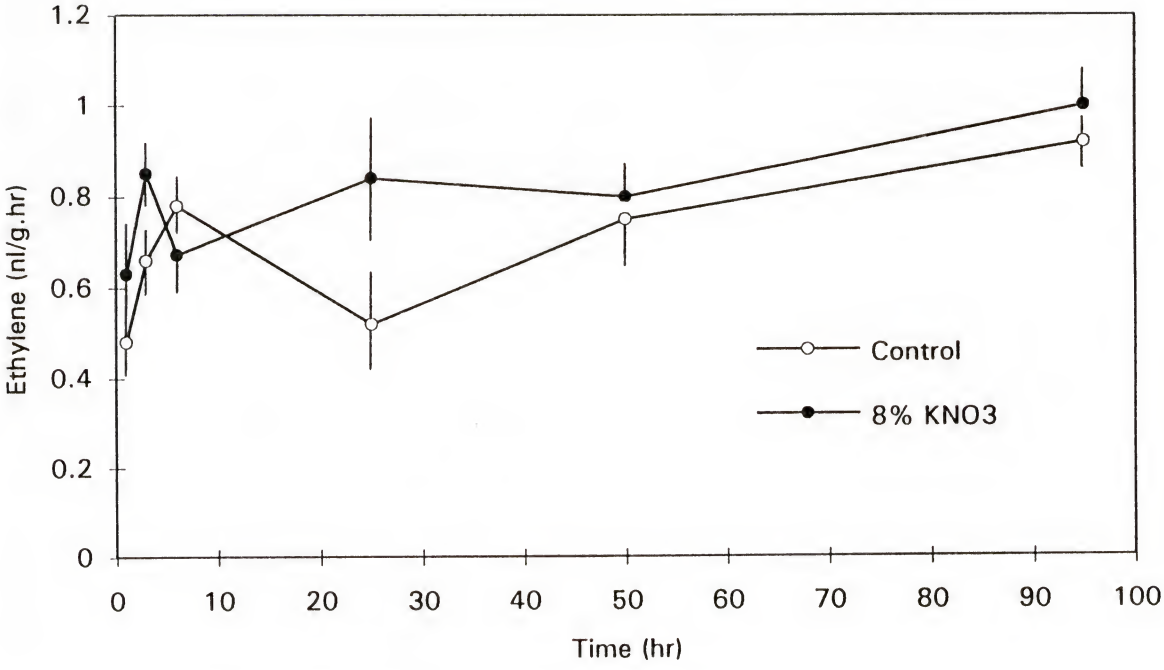

Figure 4-1. Effect of 8% KNO_3 on ethylene production by leaves during the inductive period. Branches were sprayed in December, 1988. Leaves were collected at various time intervals. Each datum is the average of 10 samples; each sample consisted of 3 leaves.

control branches flowered profusely during the same time period.

Ethylene Production During the Nonfloral-inductive Period in Response to Chemical Sprays (Single Leaf Sampling)

Ethylene production by leaves treated with 2 or 8% KNO_3 during the noninductive period was extremely low and notsignificantly greater than that of water-sprayed, control leaves, during the 98-hr time-course (Fig. 4-2). In contrast, all ethephon treatments significantly enhanced leaf ethylene synthesis over the control in the same time period (Fig. 4-3). All treatments were ineffective in stimulating flower formation in the experimental branches from which leaves were collected.

Ethylene Production During the Nonfloral-inductive Period in Response to Chemical Sprays (Periodic Leaf Sampling)

Ethylene production by leaves collected periodically from branches sprayed with 8% KNO_3, 2,000 $mg \cdot l^{-1}$ ethephon, or water during the noninductive period is shown in Fig. 4-4. KNO_3-treated leaves produced no significant ethylene over the control treatment during the entire 87-hr study period. Ethephon-treated leaves produced substantial quantities of ethylene, but less than ethephon-treated leaf samples where ethylene production was monitored in the same leaves for 98 hr (Fig. 4-3).

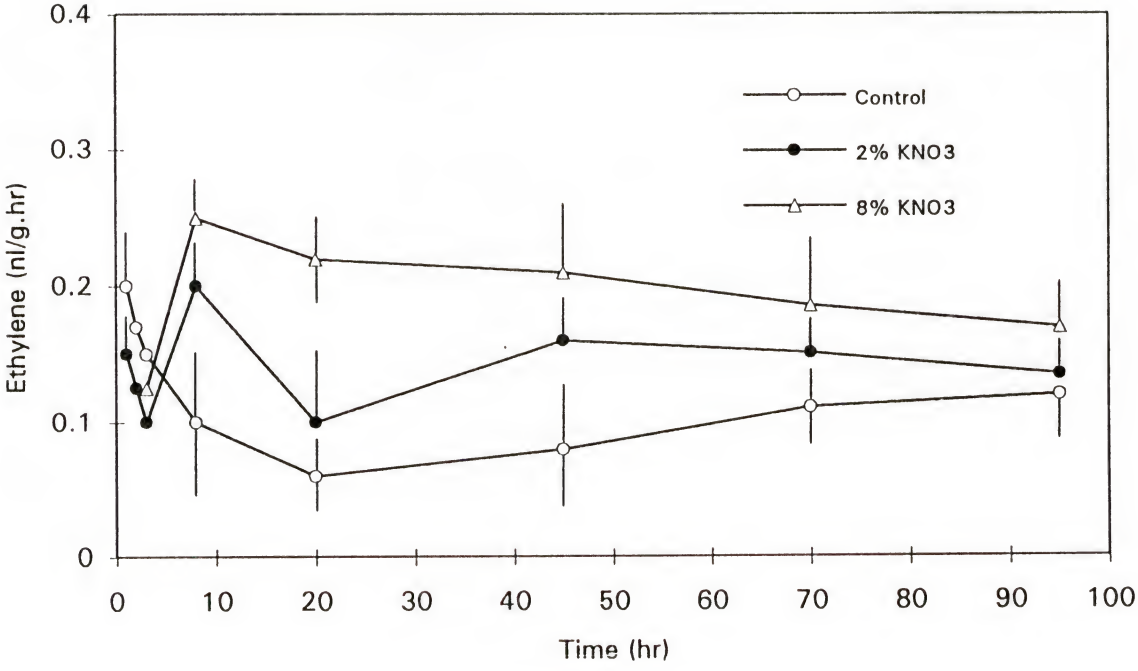

Figure 4-2. Effect of KNO_3 on ethylene production by leaves excised from branches sprayed during the noninductive period. Leaves were harvested immediately following spray application, and ethylene determined over time. Each datum is the average of 6 samples; each sample consisted of 3 leaves.

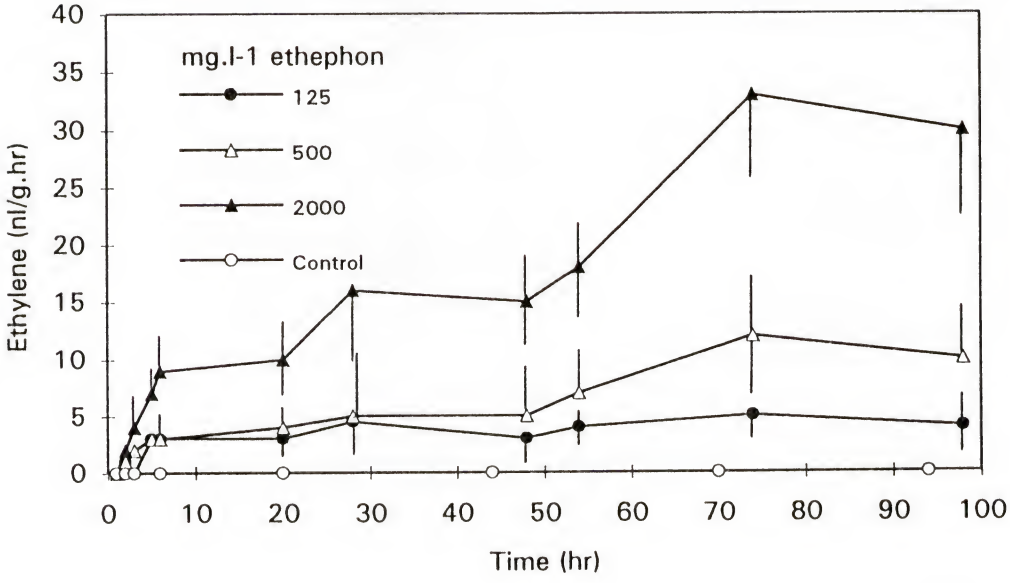

Figure 4-3. Effect of ethephon on ethylene production by leaves excised from branches sprayed during the noninductive period. Leaves were harvested immediately following spray application, and ethylene determined over time. Each datum is the average of 6 samples; each sample consisted of 3 leaves.

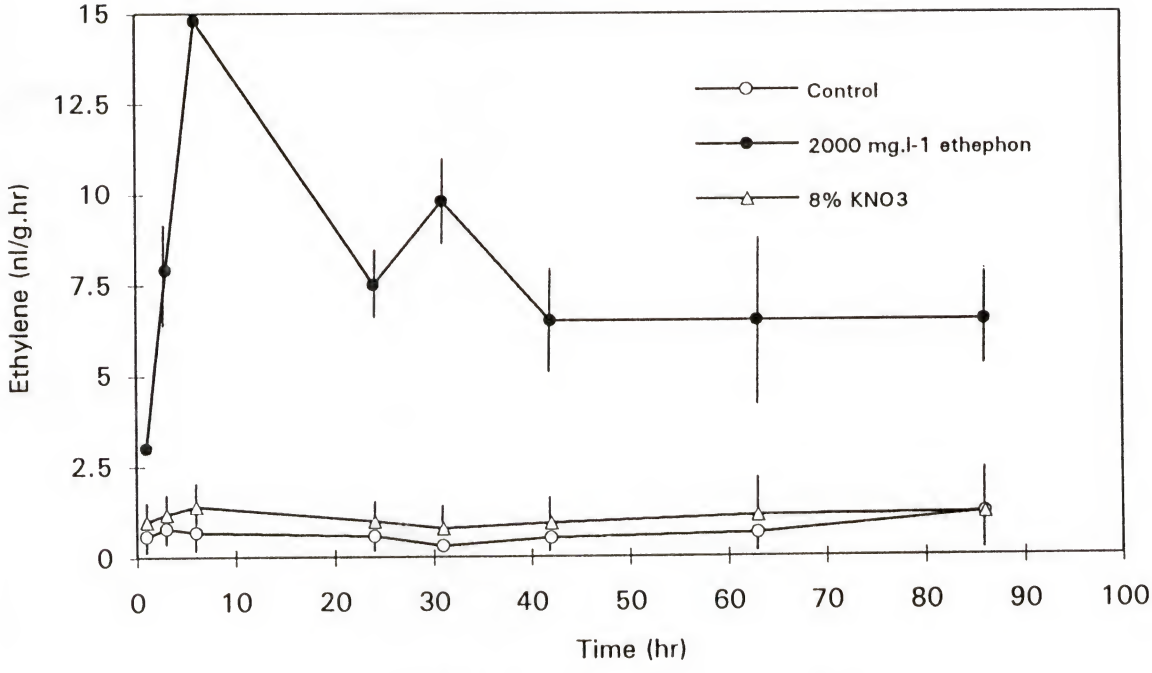

Figure 4-4. Effect of ethephon and KNO_3 on ethylene production by leaves during noninductive period. Branches were sprayed in June, 1989. Leaf samples were collected periodically for ethylene measurement. Each datum is the average of 8 samples; each sample consisted of 3 leaves.

Discussion

Results of these experiments indicate that flower initiation of 'Tommy Atkins' mango was not correlated with ethylene production in buds, leaves or shoot stems. Enhanced ethylene production was also not detected during development of vegetative buds. These results contrast with those of Saidha et al. (1983) who found that ethylene production rates increased as the flowering seasonapproached. The discrepancy may be explained by cultivar or environmental differences.

Application of KNO_3 had no influence on ethylene production either during inductive period in December (Fig. 4-1), or during the noninductive period in June (Fig. 4-2). The results of Mosqueda-Vázquez and Avila-Reséndiz (1985) indirectly suggested that the action of KNO_3 was mediated by ethylene. They employed 2% KNO_3 followed by sprays of $CoCl_2$ or $AgNO_3$ 1 to 4 hr later. However, water sprays 1 to 4 hr after spraying KNO_3 were not included to validate whether the applied KNO_3 was not washed off by the subsequent sprays of $CoCl_2$ or $AgNO_3$ solutions. The present results would suggest that the effect of KNO_3 on flowering is not mediated by ethylene.

Ethephon sprays enhanced ethylene production by the leaves (Fig. 4-3). If ethylene were responsible for floral initiation, the large amounts produced by leaves in response

produced by leaves in response to ethephon application would be expected to result in floral initiation as buds underwent growth and differentiation.

Data from the present experiments indicate that increased ethylene biosynthesis was not a pre-requisite for floral initiation in 'Tommy Atkins' mango. It was also shown that enhancing ethylene production with ethephon was ineffective in causing floral initiation. Sprays of KNO_3, which promote mango floral initiation in tropical climates, did not increase ethylene production by leaves, and showed no promotive effect on flowering of 'Tommy Atkins'. At present there is no satisfactory explanation for the lack of flowering response of 'Tommy Atkins' to chemical stimulators. Because 'Tommy Atkins' appears to experience a more profound dormancy prior to floral initiation than that seen in responsive cultivars, more potent methods of breaking dormancy need to be investigated for 'Tommy Atkins' mango.

CHAPTER 5
CORRELATIVE CONTROL OF FLOWERING: EFFECT OF GIRDLING AND DEFOLIATION

Floral morphogenesis in many herbaceous plants is induced by an unidentified floral stimulus that originates in leaves and translocates in the phloem (Bernier et al., 1981, 1993). Flowering of mango, although a woody perennial fruit tree, also involves a leaf-generated stimulus of unknown nature (Reece et al., 1946, 1949; Singh, 1959; Kulkarni, 1986; Chacko, 1991).

Mango inflorescences typically originate from apical buds of vegetative shoots. Shoot apical meristems alternate periods of activity with periods of dormancy, producing one or more vegetative flushes prior to converting to floral morphogenesis (Hallé et al., 1978). Inflorescences can also develop from axillary buds of shoots when the terminal inflorescence is detached early in the flowering season (Reece et al., 1946). Apical and axillary buds are nondifferentiated during periods of shoot dormancy, consisting only of an arrested apical meristem and several subtending, preformed nodes which consist of an axillary meristem and an inner scale (Lanuza, 1937; Mustard and Lynch, 1946; Singh, 1958; Ravishankar et al., 1979;

Scholefield et al., 1986; Sen and Mallik, 1941). Upon dormancy release, buds quickly initiate vegetative or floral primordia. Bud morphogenesis is influenced by temperature conditions. Warm temperatures near 30°C promote vegetative morphogenesis, whereas floral morphogenesis is generally promoted after a period of cool temperatures around 15°C (Shu and Sheen, 1987; Whiley et al., 1989). Differentiation of inflorescence or vegetative buds is continuous and quickly followed by budbreak (Mustard and Lynch, 1946).

Early girdling and defoliation experiments were performed by Reece et al. (1946, 1949) to examine correlative influences in flowering of 'Haden' mango. In girdled branches, vegetative shoots were decapitated near the flowering period to release dormant axillary buds and promote bud growth and differentiation. Leaves were either left attached or removed at different time intervals after girdling. Axillary buds produced inflorescences only when leaves were attached at the time of bud growth and differentiation. This occurred only when leaves were attached for 4 or more days after girdling. Axillary buds of decapitated shoots on girdled branches produced only vegetative shoots when all leaves had been removed at the time of girdling. Based on these observations, authors suggested that floral induction required ongoing cell division of bud meristems in the presence of the floral

stimulus. They also hypothesized that the floral stimulus does not trigger cell division (which was stimulated by shoot decapitation), but when such stimulus is present in sufficient amounts at the time of bud growth and differentiation, it induces the formation of floral primordia in bud meristems undergoing cell division.

The early studies of Reece et al. (1946, 1949) revealed the existence of a leaf-generated floral stimulus that was not involved in dormancy release, and whose translocation was interrupted by girdling. In the present study we examined the effect of the lag period between defoliation and branch girdling, and between girdling and defoliation, on floral initiation of 'Keitt' and 'Tommy Atkins' mango. Our purpose was to gain information on the temporal translocation pattern and the morphogenic expression of the floral stimulus in mango.

Materials and Methods

Plant Material

Experiments were initiated on blooming, 8-yr-old 'Keitt' and 'Tommy Atkins' mango trees in January, 1991. Branches measuring 1.5 to 2.0 m in length and 3 to 4 cm in basal diameter were selected in four trees. Branches (n = 4) included up to 12 flowering shoots and measured between 1.2 m and 2.0 m in length. Each shoot had 2-3 flushes of vegetative growth and terminated in an apical inflorescence.

Shoots were deblossomed (the apical inflorescence of each shoot was removed) on day 0 to release dormant axillary buds for stimulating their growth and differentiation. All shoots were deblossomed by cutting apical inflorescence at the base with pruning shears, leaving a 5-mm stump.

Effect of the Lag Period from Girdling to Defoliation on Floral Initiation

The effect of the duration of leaf attachment on axillary bud morphogenesis was examined in this experiment. Four branches on each of four trees of each cultivar were selected in January, 1991. Branches were simultaneously deblossomed and girdled at their base by removing a 5-cm-wide bark band. Branches were subsequently defoliated 0, 5, 10, or 15 days after girdling to remove the sources of floral stimulus at progressive stages of axillary bud development. Control branches were girdled and deblossomed, but not defoliated.

Effect of the Lag Period from Defoliation to Girdling on Floral Initiation

Shoots of selected branches were deblossomed as above and branches were defoliated on day 0. Branches were subsequently girdled at 0, 10, 15, 20, 25, or 30 days after defoliation. The purpose was to interrupt, at progressive stages of axillary bud development, the influx of floral stimulus produced by leaves in other sections of the canopy.

The control treatment consisted of branches that were simultaneously deblossomed and defoliated, but not girdled.

Results and Discussion

Effect of Deblossoming on Dormancy Release

All axillary buds of deblossomed shoots were nonswollen at the time of removing apical inflorescences. Axillary buds started to swell between 7 and 14 days after deblossoming. Swelling indicated the start of primordia initiation. The number of axillary buds released per deblossomed shoot ranged from 2.5 to 3.1 regardless of cultivar (data not shown). Growing buds were located immediately below the stump of the removed inflorescence.

Because bud growth commenced at about the same time among all treatments, bud morphogenesis was attributed to the availability of floral stimulus during the period of bud differentiation. The availability of floral stimulus was, in turn, influenced by the time of defoliation or girdling, as these procedures altered the production and rate of translocation of the stimulus, respectively. Cool temperatures during the experimental period were conducive to floral initiation, presumably by promoting the synthesis/action of the floral stimulus.

Effect of the Lag Period from Girdling to Defoliation on Floral Initiation

The source of floral stimulus varied depending on whether defoliation was performed after girdling or girdling

was performed after defoliation. In this experiment the floral stimulus was supplied by leaves within the deblossomed shoots, because girdling on day 0 prevented the movement of foreign floral stimulus into the experimental branches.

The two cultivars exhibited similar responses to periodic defoliation after girdling. When defoliation was performed on day 0 after girdling, all released axillary buds of deblossomed shoots of both cultivars developed into vegetative shoots (Figs. 5-1 and 5-2). This response suggests that the actively growing meristems of released buds were not supplied by floral stimulus at the time of primordia initiation. When defoliation was performed 5 days after girdling, only 2% ('Tommy Atkins') and 17% ('Keitt') of deblossomed shoots produced axillary inflorescences. Since buds were not swollen at the time of defoliation, it is deduced that floral stimulus still remained in stem tissues of deblossomed shoots when buds underwent differentiation a few days later. Although the presence of cool temperatures may have extended the viability of floral stimulus remaining after defoliation, production of vegetative shoots was greater than that of inflorescences. This response suggests that the residual floral stimulus was nearly extinguished at the time of bud differentiation.

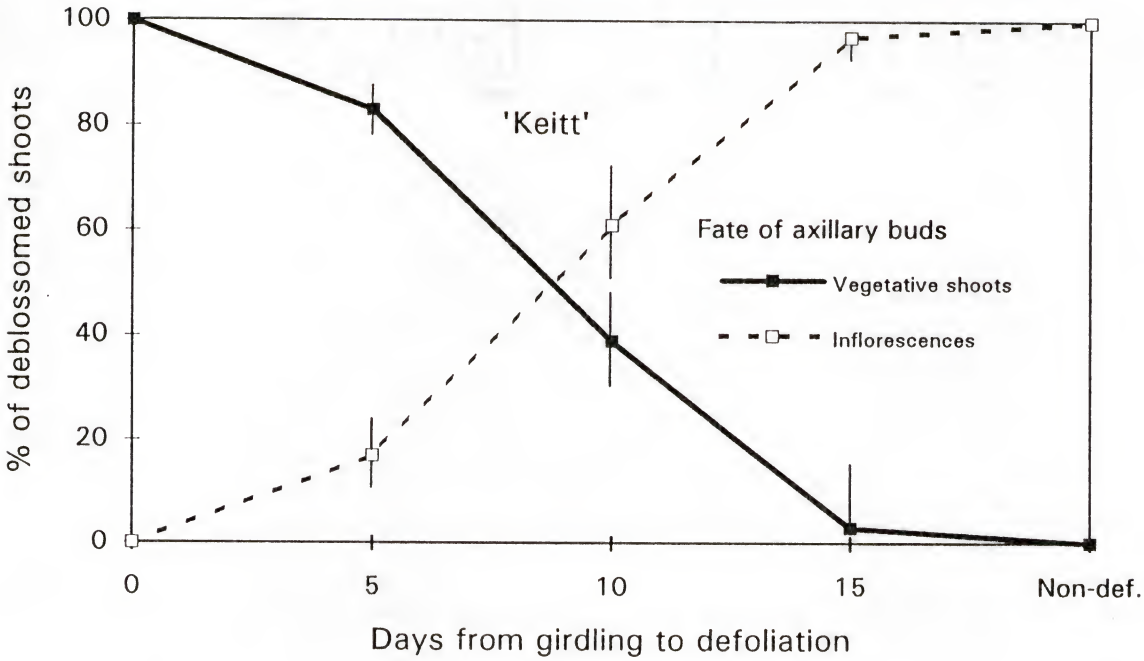

Figure 5-1. Effect of the lag period between girdling and defoliation on the developmental fate of axillary buds of 'Keitt' mango. Experimental shoots with apical inflorescences were deblossomed to stimulate bud growth and differentiation of axillary buds. Each data point represents the percentage of deblossomed shoots in which axillary buds developed into vegetative shoots or inflorescences. Each treatment was tested on 4 branches in separate trees. Each branch had at least 30 individual deblossomed shoots. Data correspond to day 45 after starting treatments.

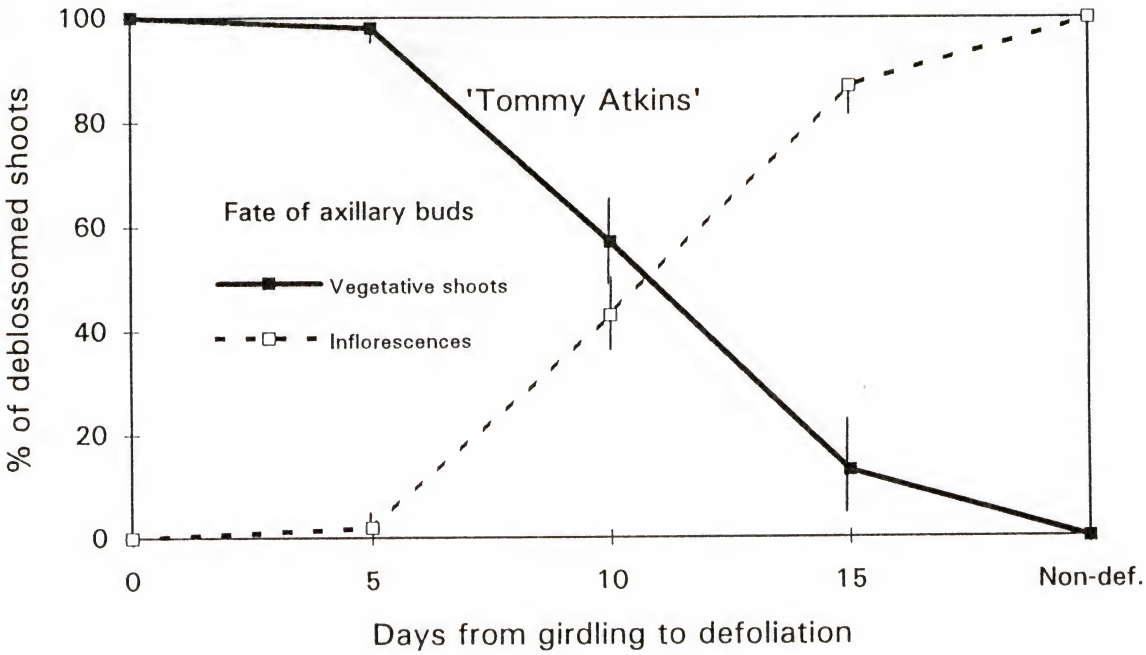

Figure 5-2. Effect of the lag period between girdling and defoliation on the developmental fate of axillary buds of 'Tommy Atkins' mango. See Figure 5-1 for details.

The amount of deblossomed shoots producing axillary inflorescences increased sharply when defoliation was performed 10 or 15 days after girdling (Figs. 5-1 and 5-2). In 'Keitt', 61% and 97% of deblossomed shoots produced axillary inflorescences when leaves were left attached for 10 or 15 days, respectively. In 'Tommy Atkins', 43% and 87% of deblossomed shoots produced axillary inflorescences when leaves remained attached for 10 and 15 days, respectively. Most buds grew while leaves were still attached or shortly after defoliation, while residual floral stimulus was presumably still viable.

All deblossomed shoots produced axillary inflorescences when leaves were retained, indicating that bud differentiation occurred in the presence of floral stimulus. As a rule, buds that were differentiating at the time of defoliation developed into inflorescences. Thus, bud release in the presence of inductive leaves supplying floral stimulus, and cool temperatures permissive to the activity of the stimulus, favored initiation of floral primordia. Results of these experiments agree closely with those reported by Reece et al. (1946, 1949) for 'Haden' mango. Our results further suggest differences in the flowering potential among mango cultivars because for any girdling-to-defoliation lag period tested, the proportion of deblossomed shoots producing axillary inflorescences in relation to

those producing axillary vegetative shoots was higher for 'Keitt' than 'Tommy Atkins'. This could indicate that the potency or bud sensitivity to the floral stimulus is greater in 'Keitt' than 'Tommy Atkins'.

Effect of the Lag Period from Defoliation to Girdling on Floral Initiation

In this experiment, residual floral stimulus was presumably available for a few days after leaves were removed on day 0. In addition, arrival of the floral stimulus from leaves outside the experimental branches or its interception was controlled by girdling branches at different times after defoliation. Responses were similar for both cultivars. Most deblossomed shoots produced axillary vegetative shoots when branches were girdled 0, 10 or 15 days after defoliation (Figs. 5-3 and 5-4). Bud development in these cases occurred after residual floral stimulus had been metabolized, and before a pulse of floral stimulus arrived into the experimental branches.

Flowering occurred on two different types of axillary structures. One type consisted of normal inflorescences, whereas the second type of shoot contained a vegetative basal portion and a floral distal portion. The latter structures were designated vegetative-to-floral (V-F) transition shoots. Such transition shoots are peculiar structures occurring in several mango cultivars. They

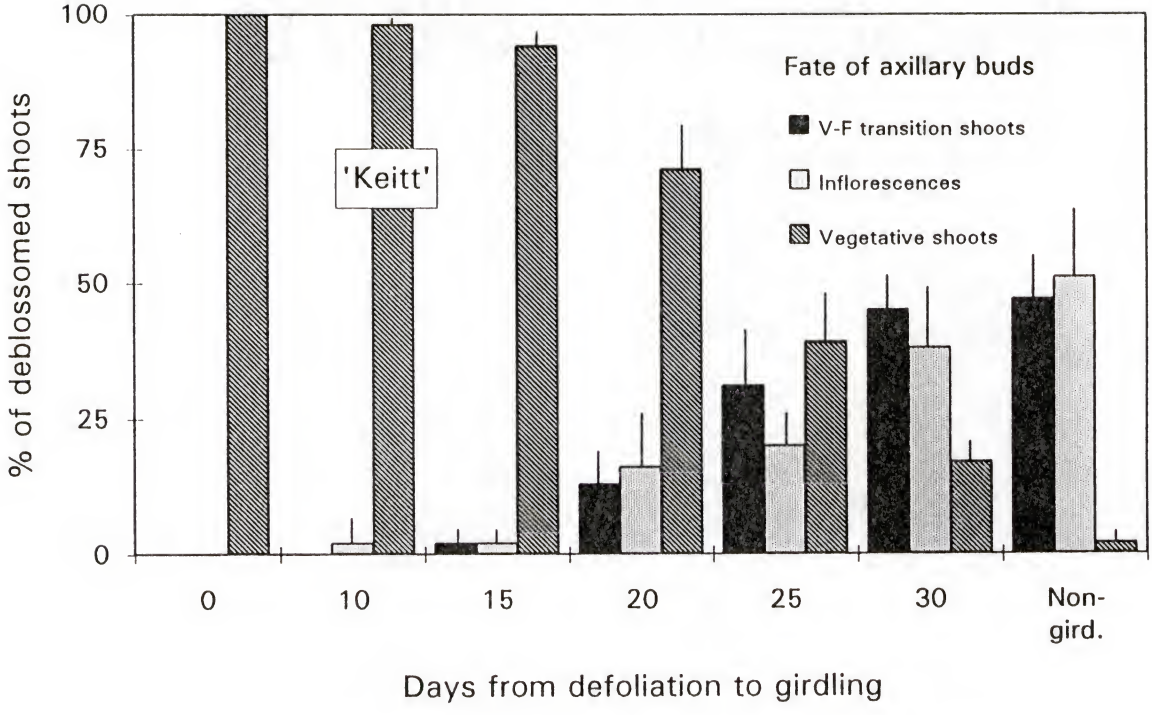

Figure 5-3. Effect of the lag period between defoliation and girdling on the developmental fate of axillary buds of 'Keitt' mango. Flowering shoots within experimental branches were deblossomed (the apical inflorescence was removed) to activate dormant axillary buds. Each data point represents the percentage of deblossomed shoots whose axillary buds produced new vegetative shoots, inflorescences, or vegetative-to-floral "transition" shoots. Each treatment was applied to 4 branches; each branch had at least 32 individual deblossomed shoots.

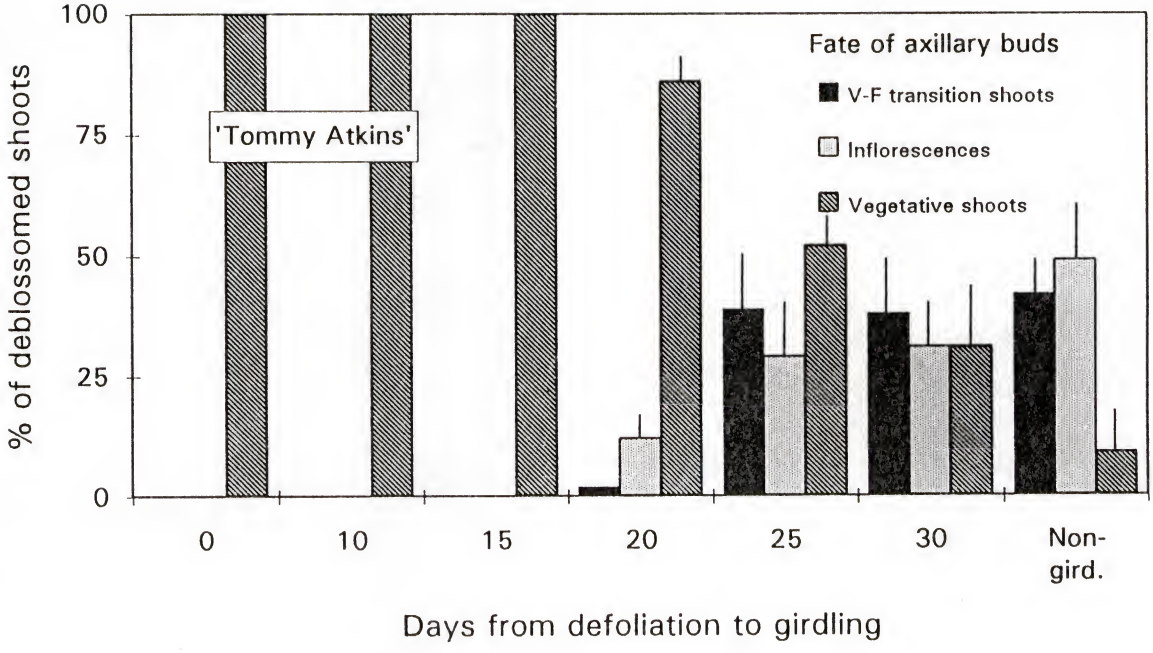

Figure 5-4. Effect of the lag period between defoliation and girdling on the developmental fate of axillary buds of 'Tommy Atkins' mango. See Figure 5-3 for details.

consist of a vegetative basal portion whose leaves contain an arrested axillary bud and a purely floral, distal portion resembling that of a typical generative inflorescence. An intermediate zone in which leaf axils have developed into a floral cluster may or may not be present. Transition shoots formed in this experiment were fully developed 7-8 weeks after bud break and did not display the characteristic constriction that delimits two separate flushes of growth. Their morphology suggests that only final stages of bud development occurred in the presence of the floral stimulus. Transition shoots comprised a vegetative and a floral morphogenic phase expressed in a single flush of growth. It is possible that the first phase occurred in the absence of floral stimulus and therefore resulted in vegetative morphogenesis. During the second phase, floral stimulus from distant leaves presumably translocated towards the developing axillary buds, causing floral morphogenesis. Growth flushes displaying transitional morphogenesis, including vegetative-to-floral, were produced in subsequent experiments (Chapter 7) by imposing temperature shifts during early bud differentiation (see Fig. 7-1).

The proportions of deblossomed shoots producing axillary inflorescences and transition shoots increased with defoliation-to-girdling lag periods of 25 and 30 days, but were slightly higher in nongirdled branches. This response

suggests that influx of floral stimulus into experimental branches peaked shortly after day 30.

To summarize, axillary buds of deblossomed shoots were released within 14 days after apical inflorescences were removed. Dormancy release in response to deblossoming was synchronous regardless of defoliation or girdling, or the time at which these operations were performed. Formation of axillary inflorescences was attributed to the presence of floral stimulus during bud differentiation. Floral differentiation occurred with girdling-to-defoliation intervals of 5, 10, or 15 days, or when leaves were not removed, and with defoliation-to-girdling intervals of 20 or 30 days, or when branches were not girdled. When branches were girdled on day 0, floral initiation was due to the presence of leaves on the deblossomed shoots. Axillary buds produced inflorescences when they underwent differentiation in the presence of leaves or while residual floral stimulus was present in stem tissues after defoliation. When branches were defoliated on day 0, floral initiation was presumably induced by a pulse of floral stimulus arriving into the experimental branches before girdling was performed. Arrival of floral stimulus after buds resumed growth resulted in structures comprising basal vegetative morphogenesis and distal floral morphogenesis.

CHAPTER 6
EFFECT OF LEAF AGE AND DURATION OF COOL TEMPERATURE TREATMENT ON FLOWERING

Floral initiation in mango is the result of complex interactions between shoot developmental stage and environmental conditions, with leaves playing a critical morphogenic role in the process. Phenological (Cull, 1987) and physiological models (Chacko, 1991; Kulkarni, 1991; Davenport, 1993) of vegetative growth and flowering in mango have been proposed. Flowering occurs in panicles that develop from apical buds of monopodial shoots. Shoots exhibit periodic vegetative extension or "flushing" followed by dormancy, and terminate with the transition of the shoot apical meristem to floral morphogenesis (Nakasone et al., 1955; Hallé et al., 1978; Verheij, 1986; Issarakraisila and Considine, 1991). Apical buds are visually dormant and nondifferentiated between flushing episodes, containing no differentiated, vegetative or floral primordia (Lanuza, 1937; Mustard, 1946; Reece, 1949; Scholefield et al., 1986; Singh, 1960). Vegetative shoots originate from axillary buds below the insertion of the apical panicle after fruit harvest in the summer. In south Florida, apical buds of 'Tommy Atkins' mango shoots typically produce one or two

flushes of vegetative growth during the summer-fall and terminate by converting into an apical panicle during the cool winter months.

The regulation of dormancy and its release, as well as the control of bud morphogenesis in mango, are not fully understood. Dormant buds can be released and stimulated to resume growth and differentiation by several treatments, including defoliation of shoot tips, and application of nitrogenous compounds (Cull, 1991; Singh, 1960). The cytokinin-like growth stimulant, thidiazuron (TDZ) (Wang et al., 1986) has also released mango buds from dormancy (Núñez-Elisea et al., 1990).

Transition of the shoot apical meristem to floral morphogenesis occurs after a period of ecodormancy (Lang et al., 1987) imposed by cool temperature in the subtropics and by plant water deficit in the seasonal tropics (Chacko, 1986; Whiley, 1993). Experimentally, however, only cool temperatures (about 20°C day/15°C night or lower) have induced floral initiation of mango in a consistent manner (Wolstenholme and Hofmeyr, 1985; Shu and Sheen, 1987; Whiley et al., 1989). Environmental stress is thought to promote floral induction by stimulating leaves to produce an unidentified developmental signal or "floral stimulus" (Reece et al., 1946, 1949; Singh, 1959; Chacko, 1991; Kulkarni, 1988; Davenport, 1993). Floral induction is

proposed to take place when sufficient floral stimulus is present in meristems of released buds at the start of cell division (Reece et al., 1946, 1949). The floral stimulus is thought to act as a morphogenic signal, but not to trigger cell division in dormant bud meristems (Reece et al., 1949). Information is lacking on the rates of cell division in dormant buds of mango.

Floral initiation of mango occurs in released apical buds of shoots whose leaves have expanded and attained a dark-green color; however, it is not clear at what age leaves become competent for floral induction. The time of exposure to cool temperatures required for floral induction also has not been determined. This study was conducted to examine the effect of leaf age, shoot-tip defoliation, and the duration of a cool temperature treatment on dormancy release and floral initiation in apical buds of 'Tommy Atkins' mango.

Materials and Methods

Plant Material

Trees of 'Tommy Atkins' mango propagated by air-layering as previously described (Chapter 3) were planted in 12-liter containers in a peat moss and vermiculite medium (1:1 v/v) containing 60 g of Osmocote (14N:14P:14K) and micronutrients. Uniform trees were produced prior to imposing treatments by pruning to obtain new vegetative

shoots from dormant, lateral buds on the trunk or scaffold branches. Shoots were thinned when they were about 2-3 cm long, leaving 3-5 shoots per tree. Pruning was timed to provide trees with either 2-, 4-, or 8-wk-old shoots at the time of initiating cool temperature treatments. Experimental shoots consisted only of the original flush of growth, i.e., they were nonextended. All experimental trees measured between 50 and 75 cm in height.

A separate set of 48 trees was prepared as above for an experiment involving chemical forcing of bud growth. These trees contained shoots with 8- to 10-wk-old leaves at the time chemical forcing treatments were applied.

To compare the effects of cool and warm temperatures on bud release and morphogenesis, a set of 15 air-layered trees, pruned as described above, was left outdoors under warm day/night temperatures of about 28°C/22°C and RH above 75%. These trees were not exposed to cool temperatures at any time, and their growth was observed for a 3-month period.

Leaf Characteristics

Expanding leaves of 2-wk-old shoots were light green, and about 10 to 20% of final leaf size. Leaves of 4-wk-old shoots were nearly final size, semi-flaccid, light green, and had a soft, waxy texture. Leaves of 8-wk-old shoots had attained final size, and were darker green and rigid.

Cool Temperature Treatments

A floral-inductive, cool temperature treatment consisting of 18°C day/10°C night (with a 12-h photoperiod, photosynthetic photon flux of 300 to 350 μmol m^{-2} s^{-1} at canopy level, and RH about 75%) was imposed on a different set of trees for either 20, 30, 40 or 60 days in a walk-in growth chamber. Trees had either 2-, 4- or 8-wk-old leaves at the start of cool temperature treatment. Upon initiating cool temperature treatments, trees were either left intact or leaves surrounding the shoot tips were removed to release apical bud dormancy (Chapter 6). There were, thus, eight treatments for each of the three leaf ages. Each treatment was applied to six trees, resulting in a total of 144 trees for the experiment. Each tree had at least four vegetative shoots of the corresponding age.

After imposing the different durations of cool temperature treatment, trees were transferred outdoors to the warm environment. The cumulative number of apical buds displaying visible growth (buds released from dormancy) was assessed at 5-day intervals. Dormancy release was reported as the number of days after initiating treatments, at which 50% of buds were swollen as a result of resuming growth (50% dormancy release). All apical buds resumed growth during the experiment, and at the time of budbreak they were classified as either floral or vegetative.

Chemical Bud Forcing to Estimate the Minimum Period of Cool Temperatures Required for Floral Induction

Chemical treatments were applied to accelerate bud release during exposure to continuous cool temperature treatment. The purpose was to determine if rapid bud growth soon after starting the cool temperature treatment would permit floral induction and result in inflorescence initiation. Air-layers with mature, 8- to 10-wk-old shoots were used in this experiment. Each of the following four treatments was applied to a set of 12 trees: 1) control treatment where vegetative shoots were left intact; 2) shoot-tip defoliation alone; 3) defoliation of shoot tips plus soil application of 15 g NH_4NO_3 per tree; 4) micro-application (50 μl) to apical buds of an aqueous solution of 50 $mg.l^{-1}$ thidiazuron plus 0.1% (V/V) Triton X-100. To secure floral initiation, trees remained in cool temperature conditions until all apical buds attained budbreak. Percent dormancy release (percent of growing buds) was recorded 21, 38, 50, 82 and 97 days after starting the treatments. Buds resuming growth at each time interval produced either a vegetative or floral flush (apical panicle).

Results and Discussion

Dormancy Release

Shoot tips were defoliated to separate dormancy factors from floral induction caused by exposure to cool temperatures. The number of days required for 50% dormancy

release according to leaf age is shown in Fig. 6-1. Apical buds of intact shoots generally remained dormant during exposure to cool temperatures, resuming growth after trees were removed from the growth chamber. In comparison, apical buds of defoliated shoots often resumed growth during cool temperatures. The time required for 50% dormancy release tended to increase with shoot age when cool temperatures were applied for 20 or 30 days. However, buds of 8-wk-old shoots (intact or defoliated) were released sooner than buds of 4-wk-old-shoots with 40 or 60 days of cool temperatures. At this time, dormancy of mature, 8-wk-old shoots may have been receding as buds were preparing to resume growth, whereas apical buds of 4-wk-old shoots were in a deeper dormant state while shoots advanced in the maturation process.

Defoliation of shoot tips stimulated apical bud growth in shoots of all ages, but particularly in those that were 2-wk-old. Apical buds of these young, expanding shoots were apparently not yet dormant at the time of defoliation, as 50% dormancy release was observed within only 15 days, compared with 30 to 55 days required by apical buds of older, tip-defoliated shoots. The delayed resumption of apical bud growth observed with increasing shoot age with or without tip defoliation indicates that the level of bud

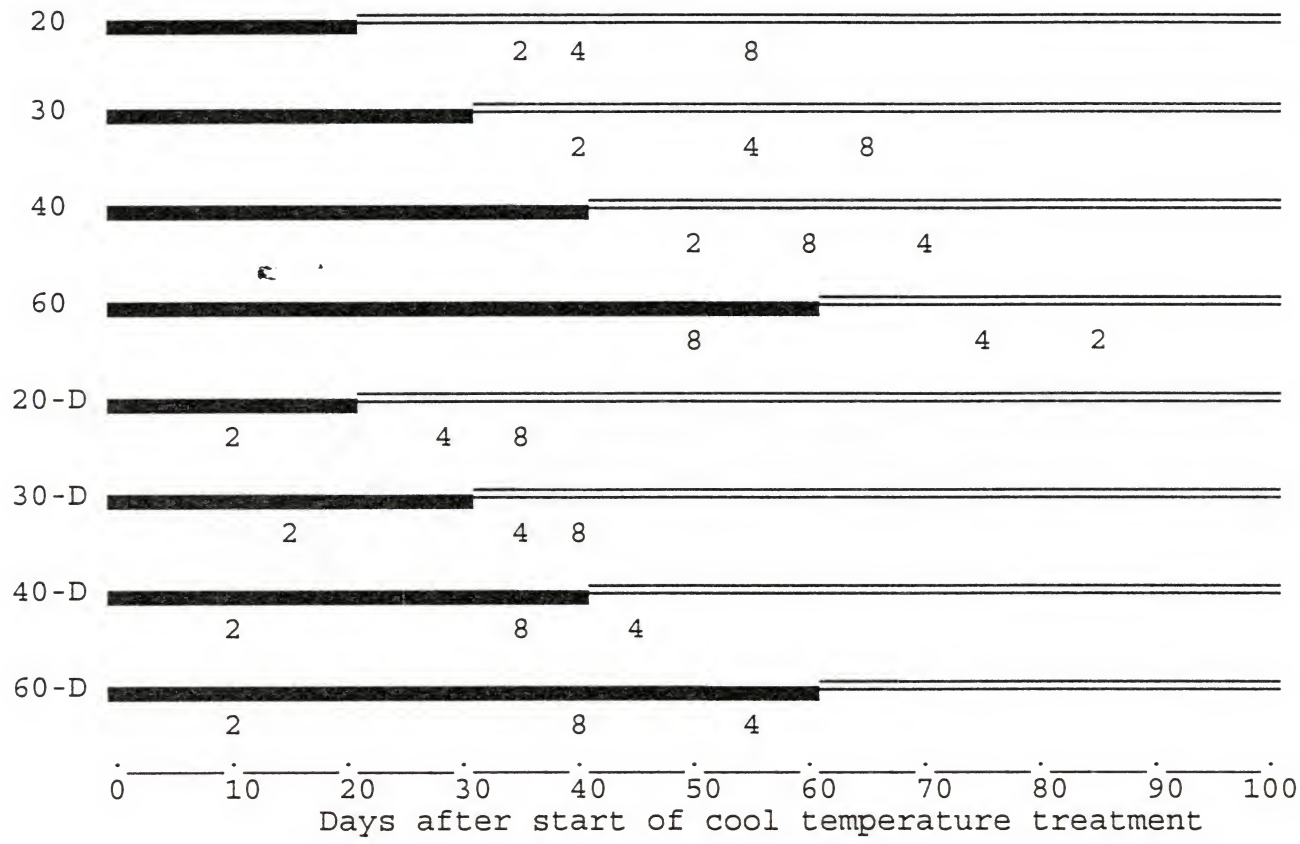

Figure 6-1. Days to 50% dormancy release (50% of apical buds exhibiting growth as evidenced by swelling) in 'Tommy Atkins' mango trees in response to cool temperatures (▬) (20, 30, 40, or 60 days at 18°C day/10°C night) and defoliation of shoot tips (D). After cool temperature treatment, trees were transferred outdoors (═) to warm temperatures (day/night temperatures ca. 28°C/20°C). Numbers below each bar indicate the day on which 50% dormancy release was recorded. Six trees having a total of at least 32 apical buds were used per treatment.

dormancy increased with leaf age. The experiment showed that defoliation of shoot tips activated growth of apical buds; however, the nature of the endogenous signal(s) releasing dormancy in natural conditions remains unclear.

Floral Initiation

Flowering occurred in trees subjected to cool temperatures (Table 6-1), but not in trees remaining in warm conditions at day/night temperatures of about 28°C/22°C, where all shoots flushed vegetatively within the 3-month observation period (data not shown). In other mango cultivars, warm temperatures above 25øC also were not conducive to floral initiation, but promoted vegetative extension of shoots (Wolstenholme et al., 1985; Shu and Sheen, 1987; Whiley et al., 1989).

In general, apical buds of developing, 2-wk-old shoots produced a vegetative flush whether bud growth resumed during or after exposure to cool temperature treatment (Fig. 6-1 and Table 6-1). Sixty days of cool temperature treatment caused only a slight floral response (8% of buds). Leaves of 2-wk-old shoots in the 60-day cool temperature treatment were nearly 8 weeks old when floral initiation began. By that time, the level of the floral stimulus was apparently sufficient to cause floral induction.

Apical buds of intact or defoliated, 4-wk-old shoots that were exposed to 20 or 30 days of cool temperatures

Table 6-1. Effect of leaf age and duration of a cool temperature treatment (18°C day/10°C night), on the type of morphogenesis exhibited by apical buds of 'Tommy Atkins' mango trees growing in containers. Apical buds produced either a vegetative or floral flush (inflorescence). Eight sets of trees (n = 6) having shoots with 2-, 4- or 8-wk-old leaves (a total of 144 trees) were subjected to four cool temperature periods. Shoot-tips were or were not defoliated to stimulate growth of apical buds. Trees were transferred to warm temperatures (day/night temperature about 28°C/22°C) after completing the corresponding cool temperature period (see Fig. 6-1).

Days of cool temperature	Bud morphogenesis (% of apical buds ± SE)					
	2-wk-old leaves		4-wk-old leaves		8-wk-old leaves	
Intact shoots	Vegetative	Floral	Vegetative	Floral	Vegetative	Floral
20	100	0	100	0	100	0
30	100	0	100	0	91 ± 1.2	9 ± 1.0
40	100	0	97 ± 0.8	3 ± 0.5	84 ± 0.9	16 ± 1.7
60	92 ± 0.5	8 ± 0.8	85 ± 2.1	15 ± 1.9	36 ± 1.8	64 ± 3.1
Def. shoots						
20	100	0	100	0	100	0
30	100	0	100	0	76 ± 2.5	24 ± 1.8
40	100	0	91 ± 0.7	9 ± 0.9	28 ± 1.6	72 ± 3.9
60	100	0	79 ± 1.3	21 ± 1.4	7 ± 0.9	93 ± 2.6

produced a vegetative flush, and did not initiate flowering even in those cases in which bud growth resumed during exposure to cool temperatures (data not shown). Few intact, 4-wk-old shoots initiated floral morphogenesis with 40 or 60 days of cool temperatures (3% and 15% of buds, respectively). In each case, tip-defoliation increased the proportion of shoots converting to flowering by 6%. Although more 4-wk-old shoots flowered compared to 2-wk-old shoots, the maximum proportion of shoots converting to floral morphogenesis was only 21%, and occurred when shoots were defoliated and exposed to 60 days of cool temperatures. It is possible that leaf aging involves an increase in floral stimulus concurrent with a decrease of floral inhibitors (Bernier et al., 1981), in particular gibberellins (Pharis and King, 1985), whose levels have been reported to decrease with leaf maturation in 'Irwin' mango (Chen, 1987).

Apical buds of 8-wk-old shoots readily initiated flowering in response to cool temperatures; however, a minimum exposure time to cool temperatures was necessary to achieve floral induction. Apical buds of intact shoots were still dormant on day 20 of cool temperature treatment, and produced a vegetative flush when trees were transferred to warm conditions (Table 6-1, Fig. 6-2). Floral initiation had occurred in 9% of shoots on day 30 after starting the

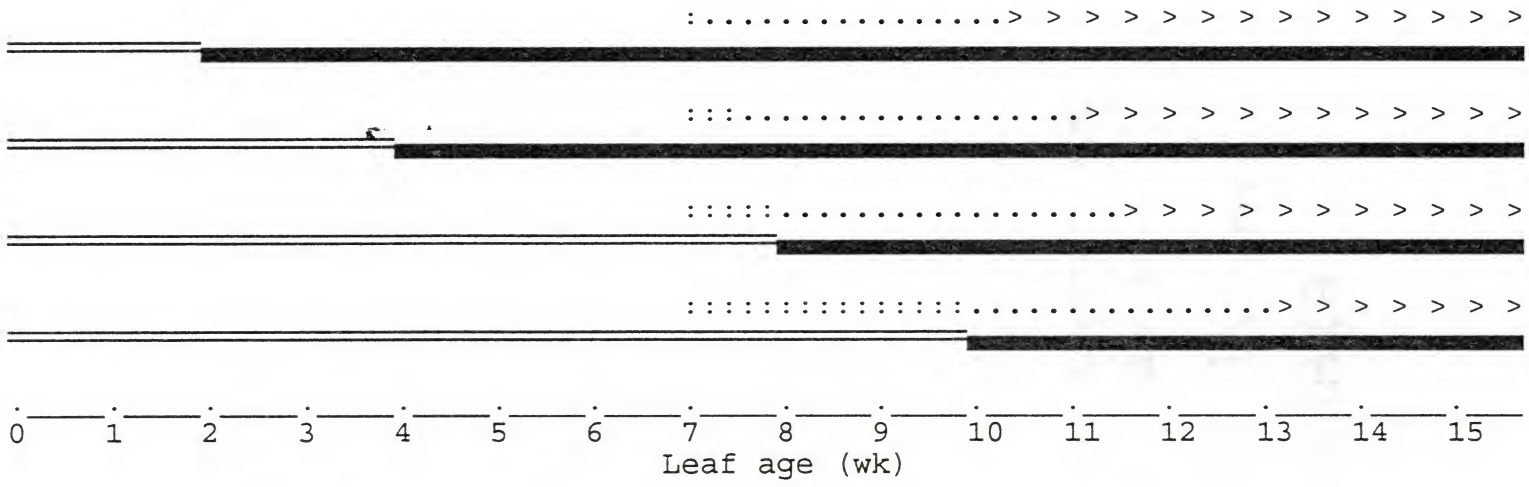

Figure 6-2. Summary of events preceding floral initiation in 'Tommy Atkins' mango trees in this study as a function of leaf age and cool temperature treatment. Containerized trees were initially pruned and grown outdoors in warm temperatures (═══) to generate new vegetative shoots. Day 0 indicates the time of emergence of vegetative shoots from lateral buds. Trees were transferred to 18°C days/10°C nights (▬▬) when leaves were 2-, 4-, 8-, or 10-wk-old. Shoot expansion and maturation occurred during the first seven weeks. Leaves became competent for responding to cool temperatures and become floral inductive upon reaching about seven weeks of age (:::; see page 81). About three weeks of exposure to cool temperatures (..) were required for bud floral induction. Induced buds remained dormant until bud growth resumed naturally or in response to defoliation of shoot tips. Floral initiation (>>>) began when induced buds resumed growth in the presence of cool temperatures.

cool temperature treatment, and the response was greater for trees exposed to 40 days (16% of shoots) or 60 days of cool temperatures (64% of shoots). The flowering trend was similar for defoliated shoots; however, defoliation caused more apical buds to resume growth during exposure to cool temperature and resulted in 24%, 72%, and 93% of apical buds initiating flowers in trees exposed to 30, 40 or 60 days of cool temperatures, respectively. Although more apical buds of defoliated shoots flowered compared to buds of intact shoots, it is assumed that floral induction had also taken place in the dormant buds of intact shoots. Interestingly, such induced buds initiated floral morphogenesis only when they resumed growth in the presence of cool temperatures, suggesting that warm temperatures inhibited floral initiation of induced buds.

Defoliation of shoot tips caused buds to resume growth during exposure to cool temperatures at a time (about 30 days into the cool temperature treatment) when leaves had produced sufficient floral stimulus (and/or the level of floral inhibitors was lower than the level of floral stimulus) to elicit floral induction. Defoliation, therefore, increased the proportion of apical buds initiating floral morphogenesis by causing more buds to resume growth during exposure to cool temperatures.

Chemical Bud Forcing to Estimate the Minimum Period of Cool Temperatures Required for Floral Induction

The preceding experiment showed that the shortest exposure to cool temperatures required for floral initiation was 30 days, imposed on 8-wk-old shoots (Table 6-1). The question arose, therefore, of whether forcing apical bud growth of mature shoots before day 30 would expedite floral initiation. The purpose of this experiment was to chemically force apical buds to resume growth in cool temperatures in less than 30 days from starting the cool treatment. The type of morphogenesis exhibited by buds resuming growth at different time intervals after starting the cool temperature treatment would approximate the duration of exposure to cool temperatures required to achieve floral induction. Shoot defoliation in combination with NH_4NO_3 or TDZ caused buds to resume growth more rapidly than defoliation alone (Table 6-2). On day 21 of cool temperature treatment, more than 70% of apical buds of defoliated shoots treated with NH_4NO_3 or TDZ displayed active growth, whereas apical buds of control shoots were still dormant. Only 6% of apical buds of defoliated shoots had resumed growth at this time. On day 50, nearly all apical buds of defoliated shoots (alone or plus NH_4NO_3 or TDZ) had resumed growth, compared with only 13% of buds on control, nondefoliated shoots. Only 52% of buds of control

Table 6-2. Effect of shoot-tip defoliation and chemical growth promoters on bud dormancy release of containerized 'Tommy Atkins' mango trees subjected to cool temperature treatment (18°C day/10°C night, 12-h photoperiod). Data refer to bud release without separating the vegetative or floral fate of released buds. NH_4NO_3 was applied at the rate of 15 g per tree, whereas a solution of 50 $mg \cdot l^{-1}$ thidiazuron (TDZ) was applied to individual apical buds at the rate of 50 μl per apical bud. Each treatment was tested on 12 trees (at least 62 apical buds).

Forcing treatment	Cumulative dormancy release (% of buds ± SE)				
	21 d	38 d	50 d	82 d	97 d
Control (nondefol.)	0	9 ± 1.9	13 ± 2.0	52 ± 3.3	95 ± 0.8
Defoliation	6 ± 1.8	79 ± 2.2	94 ± 1.3	98 ± 0.9	98 ± 0.9
Def. + NH_4NO_3	77 ± 4.1	95 ± 1.3	97 ± 1.5	97 ± 1.5	97 ± 1.5
Def. + TDZ	74 ± 3.9	91 ± 1.1	98 ± 1.4	98 ± 1.4	98 ± 1.4

shoots displayed active apical buds on day 82 of treatment. An additional 15 days in the cool temperature treatment were needed for 95% of apical buds on control shoots to resume growth.

The total amounts and proportions of apical buds initiating floral morphogenesis during each of five time intervals are shown in Table 6-3. The data indicate that the time at which buds were released from dormancy after starting the cool temperature treatment strongly influenced whether buds continued vegetative morphogenesis or shifted to floral development. Apical buds of intact or defoliated-only shoots were mostly dormant on day 21, but most buds that were further forced with NH_4NO_3 or TDZ were swollen at this time. Although more than 74% of buds resumed growth in response to NH_4NO_3 or TDZ, only 10 and 24% of these buds, respectively, initiated flowering. Most buds (85% to 100%) that resumed growth after about the fourth week of starting the cool temperature treatment initiated flowering. These results show that the primary effect of NH_4NO_3 and TDZ was to release bud dormancy, not to induce floral morphogenesis. Apical buds that resumed vegetative growth instead of initiating flowering despite adequate leaf age and exposure to low temperatures may have resumed growth with insufficient floral stimulus and/or high levels of leaf-derived floral inhibitors.

Most apical buds initiated floral morphogenesis when growth resumed after exposure to 22-38 days of cool temperature treatment. In contrast, buds continued vegetative development when growth was resumed before completing this period. Therefore, it is deduced that floral induction was achieved after a minimum of 22 days of cool temperature treatment. These results agree with those obtained in the previous experiment, in which apical buds of 8-wk-old shoots did not initiate flowering unless bud growth resumed about 30 days into the cool temperature treatment (Table 6-1). It is inferred that about three weeks of cool temperature treatment resulted in floral induction (Fig. 6-2) as a result of activation of the leaf-generated floral stimulus. Although floral induction is proposed to take place at the time of cell division (Reece et al. 1946, 1949), it is also possible that bud meristems are exposed to the floral stimulus and induced (programmed) for floral morphogenesis prior to commencing cell division, remaining dormant until the factor(s) triggering cell division become available. The rates of cell division in visually dormant buds of mango have yet to be determined in order to establish the timing of floral induction in relation to meristematic cell division.

The results on the time of exposure to cool temperatures required for floral induction are not

consistent with those of Shu and Sheen (1987), who reported that floral induction in axillary buds of 'Haden' mango was achieved after as few as four days of low temperature treatment (19°C day/13°C night, 12-h photoperiod). In that study, experimental trees had recently produced apical panicles as a result of exposure to low winter temperatures outdoors. The trees were transferred to the cool temperature regime and deblossomed to release subtending axillary buds. The time required for floral induction was considered to be the time required for released axillary buds to reach about 4 mm in length, which varied from 4 to 45 days. The study, however, did not demonstrate that axillary buds of trees initially growing in warm conditions and transferred to cool temperatures required only 4 days to be capable of initiating floral development, and that comparable axillary buds of trees remaining in warm temperatures produced vegetative growth instead of flowering. Such comparison would have allowed estimating the minimum time required by axillary buds to reach floral induction. It is probable that axillary buds in that study were already induced at the time of initiating the cool temperature treatment as a result of the previous exposure of trees to low temperatures outdoors. It appears, therefore, that the study assessed the time required in cool temperatures for release of florally-induced buds rather

than the time in cool temperatures required for floral induction. The large variability observed in bud release could have been due to variation in tree size, leaf age and the number of vegetative flushes comprising each experimental shoot (data not reported).

Estimation of the Minimum Age at which Leaves Respond to Cool Temperatures

The present data show that the competence of leaves for floral induction increased with age. Chemical bud-forcing demonstrated that floral initiation occurred only after trees had been subjected to at least three weeks of cool temperature treatment (Table 6-3). On the other hand, the youngest leaf age at which floral initiation was observed was 68 days, or 4-wk-old (28-day-old) leaves that had been exposed to 40 days of cool temperature treatment (Table 6-1). Subtracting the 21-day period required for floral induction from 68 days (the youngest leaf age at which floral primordia were observed), gives 47 days or about 7 weeks, which represents the minimum age at which leaves began to respond to cool temperatures to become floral inductive.

Requirement of Bud Growth During Cool Temperatures for Floral Initiation

Attainment of floral induction did not assure initiation of floral morphogenesis. Growth of induced buds

Table 6-3. Progressive dormancy release and floral initiation in 'Tommy Atkins' mango trees exposed to a low temperature treatment (18°C day/10°C night, 12-h photoperiod). Shoots had 8- to 10-wk-old leaves. NH_4NO_3 was applied at the rate of 15 g per tree, whereas a solution of 50 $mg \cdot l^{-1}$ thidiazuron (TDZ) was applied to individual apical buds at he rate of 50 μl per apical bud. Each treatment was tested on 12 trees (at least 62 apical buds).

Forcing treatment	Percent of buds resuming growth[a]										
	0-21d	%FB[b]	22-38d	%FB	39-50d	%FB	51-82d	%FB	83-97d	%FB	Total %FB
Control (nondefol.)	0	0	9	85	4	100	39	89	43	98	89a[c]
Defoliation	6	0	73	90	15	98	4	100	0	0	84a
Def. + NH_4NO_3	77	10 [d]	18	100	2	100	0	0	0	0	28b
Def. + TDZ	74	24 [d]	17	100	7	100	0	0	0	0	42b

[a] As evidenced by bud swelling.
[b] Proportion of buds that initiated floral morphogenesis; remaining released buds produced a vegetative flush.
[c] Means with different letters are statistically different according to Duncan's multiple range test ($P = 0.05$).
[d] More than half of these buds generated both leaves and flowers, suggesting a fractional floral induction.

in the presence of cool temperatures seemed necessary for floral initiation, because induced buds that resumed growth after trees were transferred to warm temperatures outdoors produced a vegetative flush instead of an apical panicle. Temperatures near 30°C apparently reverted floral induction, causing induced buds to continue vegetative development instead of initiating floral morphogenesis. This response coincides with that reported by Shu and Sheen (1987), in which axillary buds that were previously exposed to cool temperatures but resumed growth in warm temperatures (31°C day/25°C night) expressed vegetative instead of floral morphogenesis.

CHAPTER 7
MORPHOGENIC EFFECTS OF A TEMPERATURE SHIFT DURING BUD DIFFERENTIATION

Shoots of mango exhibit periodic vegetative extension and terminate by producing an apical inflorescence (Singh, 1960; Hallé et al., 1978; Chacko, 1986). Each episode of activity of the apical bud produces a "flush" of growth. Dormant buds contain an arrested apical meristem and preformed nodes, but no differentiated primordia. Each node consists of an axillary meristem covered by an inner scale (Lanuza, 1937; Mustard and Lynch, 1946; Ravishankar et al., 1979; Scholefield et al., 1986; Sen and Mallik, 1941; Singh, 1958).

Apical buds remain visibly dormant between flushing episodes. Upon dormancy release, bud differentiation, budbreak, and expansion of the new flush of growth are continuous. Apical buds exhibit two main developmental fates upon resuming growth and differentiation. A flush of vegetative growth (leafy shoot extension) occurs under warm temperatures about 20°C or higher, whereas inflorescence (panicle) development is initiated in cool temperatures

around 15°C (Ou, 1982; Wolstenholme and Mullins, 1982; Wolstenholme and Hofmeyr, 1985; Shu and Sheen, 1987; Whiley et al., 1989). Morphogenic effects of a sudden temperature change imposed during the course of bud differentiation, however, have not been investigated.

The first histological evidence of inflorescence differentiation in mango buds is the development of the basal axillary meristems into inflorescence primordia. Each primordium gives rise to a floral cluster (lateral flowering "branch") of the inflorescence. The inner scales of inflorescence buds remain undeveloped and later abscise during budbreak or inflorescence expansion. In buds that produce a vegetative flush, leaves arise from further differentiation and development of inner scales whose axillary meristem remains in an arrested condition (Lanuza, 1937; Singh, 1958). Mango floral development differs from that of most deciduous fruit trees, since dormant buds in the latter contain floral primordia at the time of entering dormancy, and buds remain dormant for several months before resuming development and attaining budbreak (Mustard and Lynch, 1946; Sedgley, 1990).

Although mango flowers are mainly borne on purely generative inflorescences, "leafy inflorescences" (or "mixed shoots") can also originate as a result of mild floral-inductive conditions (van der Meulen et al., 1971). These

structures originate from buds in which both the inner scale and axillary meristem of individual nodes undergo differentiation as a leaf and floral cluster, respectively (Lanuza, 1930; Scholefield et al., 1986).

The flowering process in angiosperms is thought to involve an interaction between floral-promotive and floral-inhibitory signals (Bernier et al., 1981; Schwabe, 1987). In mango, a leaf-generated floral stimulus of unknown identity is necessary for differentiation of inflorescence primordia (Reece et al., 1946, 1949; Singh, 1959; Kulkarni, 1986; Chacko, 1991), whereas floral initiation is repressed by gibberellins (Kachru et al., 1971; Chen, 1987). A period of stress imposed by cool temperatures (subtropics) or water deficit (dry tropics) typically precedes flowering (Chacko, 1991; Davenport, 1993; Whiley, 1993), suggesting that such conditions allow expression of floral-promotive over floral-inhibitory signals. The course of bud morphogenesis in mango may, thus, involve an interaction between the floral stimulus and endogenous gibberellins (Chacko, 1991, Kulkarni, 1991; Davenport, 1993).

The purpose of this study was to determine the effect of temperature modification during the process of bud differentiation in 'Tommy Atkins' mango. The morphogenic responses to a sudden temperature change imposed during the course of vegetative or inflorescence bud differentiation

were examined in apical buds of trees growing under controlled environmental conditions.

Materials and Methods

Plant Material and Environmental Conditions

Trees of 'Tommy Atkins' mango were propagated by air-layering (Chapter 3) and grown outdoors in 12-liter containers during spring-summer months. Air-layers were defoliated and pruned (leaving scaffold branches only) at the time of detachment from mother trees to generate new vegetative shoots of known, similar age. Canopies consisted of 4 to 7 vegetative shoots, each consisting only of the original flush of growth produced after pruning. Trees were exposed to temperature treatments when shoots were 8 to 10 weeks old. At this age, leaves were dark-green and fully expanded. Apical buds were nonswollen and visually dormant.

A floral-inductive, cool temperature treatment was provided in a growth chamber set at 18°C day/10°C night, 12-h photoperiod, photosynthetic photon flux of 300 to 350 μmol m^{-2} s^{-1} at canopy level, and RH > 75%. A separate growth chamber was set at 30°C day/25°C night to provide a nonfloral-inductive environment. All other environmental parameters in the warm chamber were the same as those in the cool chamber.

Microscopic Dissection

This preliminary experiment was conducted to compare bud development in the warm and cool temperature regimes and determine whether bud external appearance corresponded with stages of development of inflorescence primordia. Fifteen trees were each placed inside the warm and cool growth chambers until apical bud growth resumed. Apical buds were classified according to three distinct morphological stages. *Stage 0* buds were dormant, compacted, and covered with tightly-clasped external scales. *Stage 1* buds were swollen and had external scales that were still tightly clasped. *Stage 2* buds displayed external scales which were beginning to break open.

Apical buds (n = 12 to 15) were excised from shoots, and placed in deionized water at room temperature (26°C ± 1°C). They were dissected and observed under a stereoscopic microscope within 3 h of excision. Outer and inner scale axils were removed with a surgical scalpel to observe development of the axillary meristems of inner scales. The proportions of buds in each morphological stage displaying leafy or floral primordia were recorded.

Transfer of Trees from Warm to Cool Temperatures

Forty-nine trees with stage 0 apical buds were placed in the chamber set at 30°C day/25°C night. All shoot tips

were defoliated (6-10 leaves were removed) to activate growth and differentiation of the apical bud. Thirty nine trees were transferred from the warm condition to the cool, 18°C day/10°C night, temperature regime when bud growth had commenced (10 to 18 days after starting the high temperature treatment). Individual apical buds within each tree were classified according to the morphological stages 0, 1, and 2 described earlier. Transferred trees remained in the cool temperature regime for 57 days, a period that allowed completion of bud differentiation and the development of the new flush of growth. A set of 10 control trees was kept in the warm chamber until tagged apical buds produced a new flush of growth (15 days). Control trees were then transferred outdoors. The effect of temperature modification on bud morphogenesis was determined based on the phenotypes expressed by the apical buds of transferred and nontransferred, control trees.

Transfer of Trees from Cool to Warm Temperatures.

Forty eight trees with dormant apical buds were placed in the chamber set at 18°C day/10°C night. All shoot tips were defoliated as above to stimulate growth and differentiation of the dormant apical bud. Individual apical buds within each tree were tagged according to their morphological stage. After 42 days of exposure to the cool temperature treatment, 38 trees were transferred to the 30°C

day/25°C night temperature regime. At the time of transfer, trees contained buds in all three morphological stages described above. Transferred trees remained in the warm temperature regime for 35 days, until the flush of growth produced by apical buds had developed. A set of 10 control trees was left inside the cool chamber for 40 days. At that time, apical buds had reached budbreak and the new flush of growth was expanding. The different phenotypes expressed by apical buds were characterized to determine the effect of temperature on bud morphogenesis.

Results and Discussion

The effects of temperature on bud release and morphogenesis observed in this study were similar to those reported previously for several mango cultivars (Ou, 1982; Wolstenholme and Mullins, 1982; Wolstenholme and Hofmeyr, 1985; Shu and Sheen, 1987; Whiley et al., 1989). Continuous cool temperature treatment (18°C day/10°C night) induced floral initiation, whereas warm temperatures (30°C day/25°C night) promoted vegetative morphogenesis (shoot extension). We also found, however, that the initial course of vegetative or inflorescence morphogenesis was often reversed when differentiating apical buds were exposed to a sudden change of temperature. In addition, our results indicated that warm temperatures near 30°C apparently caused dedifferentiation of incipient inflorescence primordia that

had been initiated during the initial, cool temperature treatment.

Microscopic Dissection

Vegetative and inflorescence differentiation in apical buds of 'Tommy Atkins' mango were similar to those described for other mango cultivars (Lanuza, 1937; Mustard and Lynch, 1946; Ravishankar et al., 1979; Scholefield et al., 1986; Sen and Mallik, 1941; Singh, 1958). Stage 0 apical buds collected from either warm or cool temperature regimes were indistinct, containing a visually arrested apical meristem and inner scales with undeveloped axillary meristems.

All stage 1 buds (14 of 14) that were collected from the warm temperature regime displayed elongated scales with arrested axillary meristems. No inflorescence primordia were initiated in warm temperatures. In contrast, most stage 1 buds (12 of 15) collected from the cool chamber displayed basal nodes with swollen, mound-like axillary meristems, a feature considered the earliest indication of inflorescence formation (Mustard and Lynch, 1946).

All stage 2 buds collected from the warm environment displayed elongated scales. No growth of the axillary meristems was observed. These buds were more elongated and pointed than those collected from the cool chamber. In most stage 2 buds (13 of 15) collected from the cool temperature regime, the basal nodes contained inflorescence primordia

with a multi-lobed appearance. The apical meristem of these buds was flattened compared to that of stage 2 buds developing in warm temperatures, and was surrounded by small inner scales with undeveloped axillary meristems.

Results of this trial showed that nearly 90% of buds in stages 1 and 2 contained inflorescence primordia when differentiation occurred in cool temperatures (18°C day/10°C night). On the other hand, no inflorescence primordia were initiated when bud differentiation occurred in the warm temperature regime (30°C day/25°C night). Swelling of apical buds during exposure of trees to cool temperatures was thus considered a reliable indicator of inflorescence development.

Transfer of Trees from Warm to Cool Temperatures

More than 90% of buds in all treatments resumed growth during this study (Tables 7-1 and 7-2). All apical buds of control trees which remained in the warm temperature regime grew vegetatively (Table 7-1, Fig. 7-1A). When the trees were transferred from warm to cool temperatures, most stage 0 buds (91%) produced a flush of purely generative growth (inflorescence). Although more than 80% of stage 1 buds produced flowering, most buds (46%) produced a flush of growth displaying a "vegetative-to-floral" (V-F) transition. These growth flushes consisted of a vegetative, basal portion and a flowering, terminal portion (Fig. 7-1D)

Table 7-1. Phenotypes expressed by apical buds of container-grown 'Tommy Atkins' mango trees transferred from 30°C day/25°C night (10-18 days) to 18°C day/10°C night at different stages of bud development. Data were collected 36 days after tree transfer.

Bud stage at transfer to 18°C/10°C	Apical buds		Phenotype of growth flush (% of expressed buds ± SE)			
	Total	Expr.[a] (%)	Vegetative	V-F trans.[b]	Mixed	Inflorescenc·
Nontransferred trees (n =10)[c]	51	100.0	100.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Transferred trees (n = 39)						
Stage 0[d]	75	93.3	0.0 ± 0.0	2.9 ± 0.8	5.7 ± 1.3	91.4 ± 2.3
Stage 1[e]	66	98.5	18.5 ± 3.2	46.1 ± 5.0	32.3 ± 2.9	4.6 ± 2.2
Stage 2[f]	57	100.0	91.2 ± 4.6	5.2 ± 3.1	3.5 ± 2.2	0.0 ± 0.0

[a] Percent of total apical buds expressing a flush of growth. Nonexpressed buds remained undeveloped during the experimental period.
[b] Vegetative to floral transition, i.e., buds formed a flush of growth with a basal vegetative (leafy), and distal, floral portion.
[c] Control trees that were not removed from the 30°C day/25°C night temperature regime.
[d] Buds were dormant, with no initiated primordia.
[e] Buds were swollen, with elongating inner scales.
[f] Buds were beginning to break, displaying tips of embryonic leaves.

Table 7-2. Phenotypes expressed by apical buds of container-grown 'Tommy Atkins' mango trees transferred from 18°C day/10°C (42 days) to 30°C day/25°C night at different stages of bud development. Data were collected 39 days after tree transfer.

Bud stage at transfer to 30°C/25°C	Apical buds		Phenotype of growth flush (% of expressed buds ± SE)			
	Total	Expr.[a] (%)	Vegetative	F-V trans.[b]	Mixed	Inflorescenc·
Nontransferred trees (n = 10)[c]	53	92.5	12.2 ± 3.1	0.0 ± 0.0	4.1 ± 0.0	83.7 ± 5.0
Transferred trees (n = 38)						
Stage 0[d]	61	95.1	91.5 ± 2.3	0.0 ± 0.0	8.5 ± 2.3	0.0 ± 0.0
Stage 1[e]	77	97.4	88.0 ± 2.3	6.7 ± 3.1	5.3 ± 2.3	0.0 ± 0.0
Stage 2[f]	74	98.6	10.9 ± 4.7	9.6 ± 3.1	6.8 ± 5.7	72.7 ± 5.5

[a] Percent of total apical buds expressing a flush of growth. Nonexpressed buds remained undeveloped during the experimental period.
[b] Floral to vegetative transition, i. e., buds formed a flush of growth with a floral basal portion, and a distal vegetative (leafy) portion.
[c] Control trees were not removed from the 18°C day/10°C temperature regime (n = 53 buds).
[d] Buds were dormant, with no initiated primordia.
[e] Buds were swollen, with nascent inflorescence primordia in the axils of inner scales.
[f] Buds were beginning to break and had many lobed, inflorescence primordia.

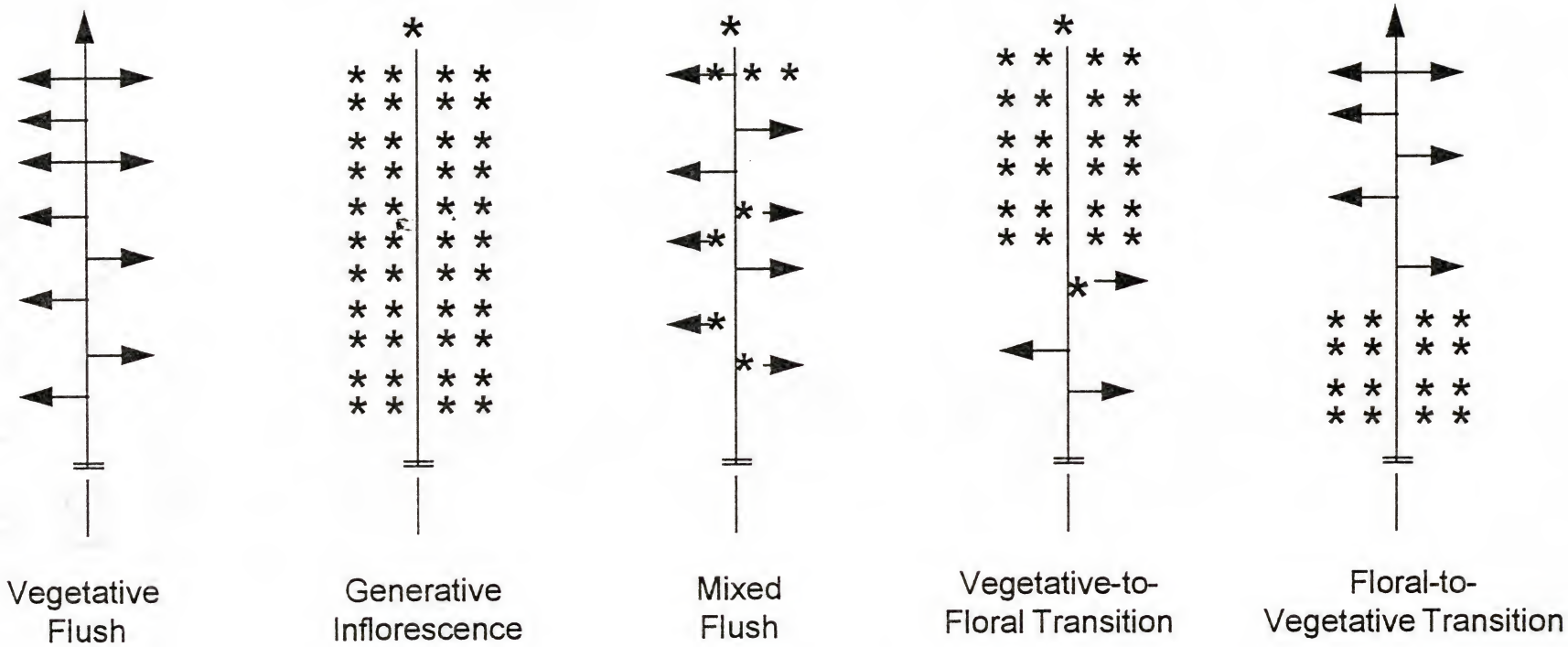

Figure 7-1. Schematic diagrams of different phenotypes of terminal flush occurring on shoots of 'Tommy Atkins' mango. The separation between the terminal and subterminal flush in each shoot is indicated by "=". In a vegetative flush, all nodes consist of leaves with dormant axillary buds (<—, —>), and an apical bud (^) is retained. All nodes of purely generative inflorescence consist of floral clusters (**) and the shoot apex has terminated by flowering (*). A mixed flush of growth has nodes consisting of leaves whose axillary bud either remained dormant (<—, —>) or produced a floral cluster (<—*, *—>), and the apex has terminated by flowering. A vegetative-to-floral transition flush has a basal leafy-mixed portion and a generative distal portion, whereas a floral-to-vegetative transition flush has a basal, generative portion and a distal, vegetative portion retaining an apical bud.

similar to that of a purely generative inflorescence (Fig. 7-1B). It is possible that in this type of transitional growth, the basal axillary meristems did not initiate inflorescence primordia, but remained in an arrested condition, due to insufficient production/activity of floral stimulus, or due to the presence of floral-inhibitory factors in warm temperature conditions. Warm conditions, however, allowed development of inner scales into leaves. Subsequent exposure of trees to cool temperatures may have permitted the synthesis or action of the floral stimulus, or caused a reduction of floral-inhibitory factors, allowing axillary meristems of the newly-formed nodes to differentiate as flower-bearing clusters, while inner scales remained undeveloped and later abscised.

Flushes of growth that underwent V-F transition briefly ceased elongating after the basal, vegetative portion was formed. The newly-formed leaves underwent expansion and maturation during this period. Visible elongation was resumed about 25 days later, and this second segment of the flush resembled the terminal portion a purely generative inflorescence. Despite the brief period of arrested elongation between the vegetative and floral portions, the flush as a whole displayed morphological continuity between the two phases. In comparison, a typical flowering shoot exhibits a marked constriction delimiting contiguous flushes

of vegetative growth and the apical inflorescence. Flushes of vegetative-to-floral transition growth were morphologically similar within and among trees in this experiment, with the proximal portion typically representing about one-third of the total length of the flush (Fig. 7-1D). The above features indicate the existence of separate morphogenic regions within buds.

In the observations with field-grown trees (Chapter 4), V-F transition shoots arising from axillary buds of decapitated shoots also completed vegetative and floral development in about five weeks and were morphologically similar to those occurring in the present experiment. It is possible that the brief period of arrested elongation preceding emergence of the floral complement involved production of the floral stimulus, or a reduction of floral-inhibitory factors in the newly-formed basal leaves, allowing the flush to undergo the transition from vegetative to inflorescence morphogenesis.

Thirty two percent of stage 1 apical buds produced a flush of mixed vegetative-inflorescence growth ("leafy inflorescences"). This type of mixed flush had leaves whose axillary buds developed into floral clusters, with the apex terminating in a small floral cluster (Fig. 7-1C). Mixed nodes possibly resulted from similar levels of both floral stimulus (Reece et al., 1946) and endogenous floral

inhibitors (Bernier et al., 1981; Schwabe, 1987; Davenport, 1993) perhaps gibberellins (Kachru et al., 1971; Chen, 1987; Chacko, 1991) at the time of primordia differentiation in the basal nodes and when the apical meristem resumed node initiation.

Nearly all stage 2 buds (91%) produced a flush of vegetative growth after trees were transferred from warm to cool temperatures (Table 7-1). In this case, vegetative differentiation during initial exposure to warm temperatures was apparently too advanced at the time of transferring trees to the cool temperature regime.

Transfer of Trees from Cool to Warm Temperatures

The flush of growth produced by most apical buds (84%) of control trees remaining in the cool temperature regime consisted of a purely generative inflorescence (Table 7-2; Fig. 7-1B). Most buds that were in stage 0 (91%) at the time of transfer from cool to warm conditions produced a flush of vegetative growth (Table 7-2; Fig. 7-1A). Thus, bud growth in the presence of cool temperatures appeared necessary for expression of inflorescence morphogenesis.

Most stage 1 buds (88%) produced vegetative growth instead of inflorescences when transferred to high temperatures. In the preliminary dissection experiment, nearly all stage 1 buds collected from trees growing in cool temperatures displayed initiating axillary primordia, which

is a characteristic of inflorescence morphogenesis. It is inferred that in this experiment, the swelling of stage 1 apical buds in cool temperatures was due to the growth of nascent inflorescence primordia. Yet, the fact that these buds produced a vegetative flush instead of an inflorescence upon transferring trees to the warm temperature regime indicates that the nascent inflorescence primordia that were initiated during exposure to cool temperatures ceased differentiation when trees were suddenly shifted to warm temperatures. This response suggests that inflorescence primordia at early stages of differentiation are not irreversibly committed (evoked) to express floral morphogenesis. Under natural conditions, it is possible that warm temperatures above a critical threshold during early inflorescence bud development cause dedifferentiation of inflorescence primordia, reducing production of inflorescences and fruiting potential of trees.

Dedifferentiation of floral primordia has been reported for other perennial fruit trees such as *Litchi chinensis* Sonn. (Menzel et al., 1989) and *Citrus sinensis* L. (Moss, 1976). As in mango, buds of these tree species begin primordia initiation and attain budbreak soon after being released from dormancy. Cessation of floral-promotive temperatures during bud development may interfere with the differentiation of floral structures. Lord and Eckard

(1987) reported dedifferentiation (reversion of floral primordia to vegetative morphogenesis) in *Citrus sinensis* L. by exogenous application of GA_3. It was suggested that high levels of endogenous gibberellins may exert a similar effect under natural conditions. Developing inflorescence primordia of mango buds cannot revert to leaf morphogenesis, as leaves and inflorescence primordia arise from different meristematic tissues; therefore, they possibly regress to a meristematic condition. Continuous temperatures near 30°C possibly inhibit the floral stimulus, but allow the activity of floral-inhibitory signals such as gibberellins.

When trees were transferred from cool to warm conditions, stage 2 inflorescence buds resulted in amounts of inflorescences similar to those produced by control trees remaining in the cool temperature regime (Table 7-2). Thus, development of stage 2 inflorescence buds was too advanced at the time of transfer to warm temperatures for interrupting differentiation of inflorescence primordia. Transfer of trees from cool to warm conditions caused stage 1 and 2 apical buds to occasionally (fewer than 10% of buds) produce a flush of growth whose basal portion contained floral clusters, and whose distal, leafy portion resembled that of a vegetative shoot (Fig. 7-1E). A flush of growth exhibiting this type of morphogenic shift was described as undergoing a "floral-to-vegetative" (F-V) transition.

Occurrence of F-V transitions suggests that during cool temperatures, the floral stimulus promoted initiation of inflorescence primordia at the axillary meristems of the basal, preformed nodes; however, no floral stimulus was available when the apical meristem resumed node production upon transfer to warm temperature conditions.

Growth flushes produced by apical buds of 'Tommy Atkins' mango exhibited distinct phenotypes as a result bud differentiation under continuous warm or cool temperatures, or a temperature shift during early or advanced bud differentiation. Results of temperature modification indicate that bud differentiation began with initiation of leaves (warm conditions) or floral clusters (cool temperatures) in the preformed nodes subtending the dormant apical meristem (Fig. 7-2). The apical meristem was activated afterwards in cool or warm temperatures, and the nodes it generated then responded to temperatures present during their differentiation. Preformed nodes and the apical meristem, therefore, respond independently to those conditions prevailing at the time of their activation.

Figure 7-3 summarizes the morphogenic responses of developing apical buds to cool and warm temperatures as a function of node position and temperature at the time of primordia initiation. It is proposed that the phenotype of

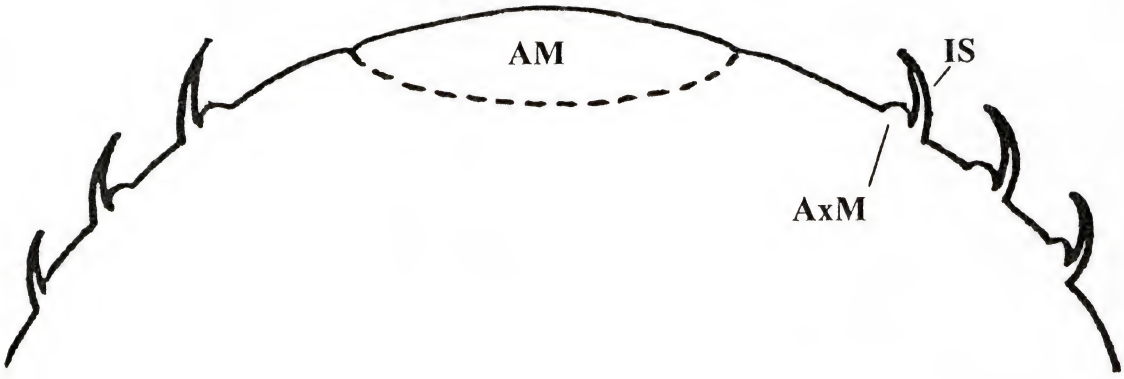

Figure 7-2. Diagram of a dormant, apical bud of mango showing the apical meristem (AM) and group of preformed nodes (each node consists of an inner scale, IS, and an axillary meristem, AxM). The apical meristem and preformed nodes respond to temperature conditions at different times. Primordia are first initiated in the preformed basal nodes. The apical meristem is reactivated later, generating a new population of nodes which undergo differentiation later. Warm temperatures (30°C day/25°C night) cause differentiation of inner scales into leaves, with axillary meristems remaining in an arrested condition. Cool temperatures (18°C day/10°C night) cause axillary meristems to differentiate into floral clusters, with inner scales developing as small bracts that abscise during budbreak or inflorescence expansion. Vegetative-to-floral transition flushes (Fig. 7-1D) formed when basal nodes developed as leaves in warm temperatures, and the apical meristem resumed node production in cool temperatures. These new nodes later developed as inflorescence primordia. Floral-to-vegetative transitions (Fig. 7-1E) occurred when basal nodes developed as inflorescence primordia in cool temperatures, and the apical meristem resumed node production later, in warm temperatures. The new nodes developed as leaves.

growth flush produced by an apical bud is determined by the temperatures in which the preformed nodes undergo primordia initiation, and subsequently, by the temperatures prevailing when the apical meristem resumes node production. Inflorescence development is promoted by cool temperatures, whereas vegetative morphogenesis is generally promoted by warm temperatures.

Conclusions

Morphogenic responses of developing apical buds of mango to continuous or shifting temperatures indicate that the apical meristem and preformed nodes behave as separate morphogenic regions within buds. The regions are activated at different times and their developmental fate appears to be regulated by temperatures prevailing during their activation. Temperatures possibly regulate the levels of floral-promotive and floral-inhibitory signals available to preformed and newly-formed nodes upon primordia initiation, directing their course of morphogenesis.

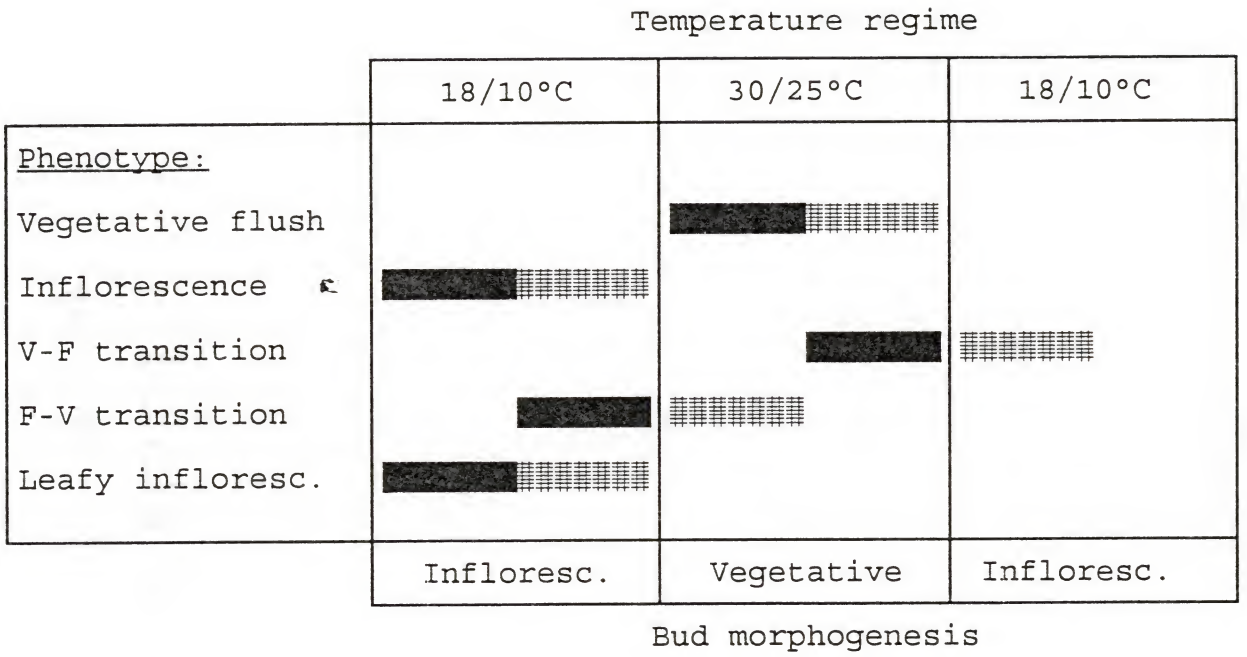

Figure 7-3. Observed morphogenic effects of temperature during apical bud differentiation in 'Tommy Atkins' mango. Cool temperatures (18°C day/10°C night) promoted initiation of inflorescence primordia, whereas high temperatures (30°C day/25°C night) promoted initiation of vegetative primordia. The bud contains basal, preformed nodes (▇) and newly-formed nodes generated upon reactivation of the apical meristem (▤). Cool or warm temperatures occurring during activation (primordia initiation) of each region resulted in an inflorescence or vegetative portion, respectively. Leafy inflorescences that were produced under continuous floral-inductive temperatures possibly resulted from sub-optimal production of the floral stimulus.

CHAPTER 8
ROLE OF PHOTOPERIOD IN FLOWERING

Mango cultivation is concentrated within 20° N and S latitudes, where the shortest annual photoperiods exceed 11 hours. In the subtropics, mango cultivars of Indian origin such as Tommy Atkins (Young and Sauls, 1981) flower during the winter months, when daylengths are shorter than 12 hours. Vegetative growth occurs during long days in the summer. Flowering in most woody perennials does not appear to be under photoperiodic control (Kozlowski et al., 1991). However for mango in subtropical conditions, it appears that short days may promote flowering, whereas flowering may be inhibited by long days. Short daylengths during the fall and winter months are often accompanied by night temperatures below 20°C, whereas long days coincide with the warmest temperatures of the year. Since cool temperatures (about 15°C) promote floral induction of mango (Chacko, 1986; Shu and Sheen, 1987; Whiley et al., 1989), the direct effect of photoperiod on mango flowering can only be assessed by experimentally separating photoperiod from temperature.

Dormant mango buds do not contain preformed, vegetative or floral primordia (Lanuza, 1937; Mustard and Lynch, 1946). Floral morphogenesis is induced by an unidentified floral stimulus that originates in mature leaves and translocates in the phloem towards apical or axillary buds (Reece et al., 1946, 1949; Singh, 1959). Floral induction is proposed to take place when a significant amount of the floral stimulus is present in bud meristems at the start of cell division (Reece et al., 1946, 1949). To separate photoperiod from temperature effects, we exposed mango trees to different photoperiods under cool (floral-inductive), or warm (nonfloral-inductive) temperature regimes. The objective was to determine if short days promote and long days inhibit mango flowering.

Materials and Methods

Mango trees (cv. Tommy Atkins) were propagated by air-layering (Chapter 3) and placed in 12-liter containers in a peat moss and vermiculite medium (1:1 v/v) during spring and summer months. Trees were pruned to generate new vegetative shoots leaving three to five shoots per tree. When shoots were 8 to 10 weeks old, trees were placed in growth chambers with 11-, 12-, or 13-h photoperiods at 18°C day/10°C night, or chambers with 11- or 13-h photoperiods at 30°C day/25°C night. Thermoperiods in the different treatments corresponded with the periods of light and darkness. A

separate treatment consisted of exposing trees to continuous light (24-h photoperiod), alternating 12-h thermoperiods of 18°C and 10°C. Photosynthetic photon flux for all treatments was 300 to 350 μmol m^{-2} s^{-1} at the upper canopy level. Relative humidity inside the chambers was above 75% at all times.

Prior to imposing treatments, some shoots had swollen apical buds, indicating the occurrence of bud growth before the treatments were imposed. To ensure bud development in response to treatments, all shoots were decapitated by clipping off the shoot apex and whorl of distal leaves at the time of enclosing trees in the growth chambers. This procedure released axillary buds from dormancy, allowing them to resume growth and undergo differentiation during exposure of trees to the experimental treatments. After removing shoot apices, decapitated shoots had between 7 and 11 axillary buds.

Trees were irrigated at 2-day intervals after enclosure in the growth chambers. Fifteen replicate trees were used per each of the six treatments in a randomized complete block design. The number of trees and proportions of axillary buds on decapitated shoots generating inflorescences or new vegetative shoots in each treatment were assessed.

Results and Discussion

Treatment effects on flowering were assessed according to the developmental fate of released axillary buds on decapitated shoots. Shoot decapitation released between 3.2 and 4.3 axillary buds per decapitated shoot and between 154 and 224 axillary buds per treatment (Table 8-1). Axillary buds within individual decapitated shoots had the same developmental fate, producing either inflorescences or new vegetative shoots.

Photoperiod had no effect on the vegetative or floral fate of axillary buds in this study, whereas temperature effects on bud morphogenesis were independent of photoperiod (Table 8-2). Cool temperatures of 18°C day/10°C night caused axillary buds to initiate floral morphogenesis (inflorescences). Warm temperatures of 30°C day/25°C night caused axillary buds to develop into vegetative shoots instead of inflorescences. Photoperiods of 11, 12, or 13 h, when combined with 18°C day/10°C night temperatures, caused floral initiation in nearly all trees and axillary buds of decapitated shoots within 40 days of imposing treatments. Continuous light with 12-h thermoperiods of 18°C and 10°C, caused flowering of nearly all trees and axillary buds within 35 days of imposing treatments. Few axillary buds developed into vegetative shoots in cool temperatures.

Table 8-1. Release of dormant axillary buds in vegetative shoots of 'Tommy Atkins' mango as a result of decapitation. Shoots were decapitated at the time of starting treatments. Each treatment was applied to 15 containerized trees. Data were collected 45 days after starting treatments.

Photoperiod (h)	Day/night temp.[z]	No. of decapitated shoots		No. of released axillary buds		
		Per tree	Total	Per shoot	Per tree	Total
11	18/10°C	3.4	51	3.6	12.3	184
12	18/10°C	3.2	48	4.1	12.8	192
13	18/10°C	3.5	52	4.3	14.9	224
24	18/10°C[y]	3.2	48	3.2	10.3	154
11	30/25°C	3.5	53	3.3	11.7	175
13	30/25°C	3.3	50	4.0	13.3	200

[z] Thermoperiods corresponded to light and dark periods.
[y] Temperature regime consisted of alternating 12-h thermoperiods at 18°C (0600 h to 1800 h) and 10°C (1800 h to 0600 h).

Table 8-2. Effect of photoperiod and temperature regimes on flowering of 'Tommy Atkins' mango. Experimental vegetative shoots were decapitated at the time of starting treatments to release dormant, nondifferentiated axillary buds. Trees remained in the growth chambers until all released axillary buds completed differentiation and reached budbreak. Data were collected 45 days after starting treatments.

Photoperiod (h)	Day/night temp.[z]	No. of flowering trees	Fate of released axillary buds (% of buds ± SE)	
			Floral[y]	Vegetative[x]
11	18/10°C	15/15	100	0
12	18/10°C	15/15	100	0
13	18/10°C	15/15	96 ± 0.4	4 ± 0.2
24	18/10°C[w]	14/15	94 ± 0.4	6 ± 0.3
11	30/25°C	0/15	0	100
13	30/25°C	0/15	0	100

[z] Thermoperiods corresponded to light and dark periods.
[y] Axillary buds that developed into inflorescences.
[x] Axillary buds that developed into new vegetative shoots.
[w] Temperature regime consisted of alternating 12-h thermoperiods at 18°C (0600 h to 1800 h) and 10°C (1800 h to 0600 h).

Photoperiods of 11 or 13 h at 30°C day/25°C night caused vegetative growth, but no flowering occurred on any tree or axillary buds of decapitated shoots. With warm temperatures, axillary buds developed into vegetative shoots within 17 days of commencing treatments. These results showed that floral initiation was regulated by temperature, but not affected by photoperiod.

In south Florida (25° N latitude), flowering of mango also occurs during short days in the winter, when photoperiods are near 11 hours, and day/night temperatures are typically below 28°/15°C. Brief episodes of higher temperatures during the winter often cause sections of tree canopies to flush vegetatively, but no vegetative flushing occurs if temperatures remain cool. Therefore, high temperatures during short, winter days cause buds to resume vegetative growth instead of initiating floral development.

In summary,, short or long photoperiods (11 or 13 h), when accompanied by warm temperatures (30°C day/25°C night), did not result in floral initiation, whereas short, long, neutral or continuous photoperiods (11, 12, or 13 h, or 24 h with 12-h thermoperiods) accompanied by cool temperatures (18°C day/10°C night) resulted mainly in floral initiation. Cool temperatures rather than a short photoperiod caused floral induction, whereas warm temperatures rather than a long photoperiod inhibited flowering in this study.

CHAPTER 9
ROLE OF WATER STRESS IN FLOWERING

Water stress affects turgor in plant cells and, consequently, influences bud growth in fruit trees. In some fruit crops, notably *Citrus* (Southwick and Davenport, 1986), water stress has the ability of inducing floral morphogenesis, with floral bud growth initiating upon rewatering.

In mango trees, floral morphogenesis is initiated during cool weather in the subtropics. Temperatures of about 15°C or lower induce floral morphogenesis, whereas temperatures of about 20°C or higher promote vegetative morphogenesis (Wolstenholme and Hofmeyr, 1985; Shu and Sheen, 1987; Whiley et al., 1989). In the tropics, mango trees flower during warm weather, after experiencing a period of drought (Beal and Newman, 1986; Chacko, 1986; Singh, 1960; van der Meulen, et al., 1971; Verheij, 1986; Whiley et al., 1989). However, floral induction of mango in response to water stress has not been demonstrated experimentally.

Mango vegetative shoots are monopodial and exhibit periodic extension or "flushing" prior to formation of the

apical panicle (Singh, 1960; Verheij, 1986). Dormant buds are nondifferentiated (Lanuza, 1937; Mustard, 1946; Reece, 1949; Scholefield et al., 1986; Singh, 1960); therefore, upon initiating growth, all buds can potentially display vegetative or floral morphogenesis. The presence of the apical bud represses growth of subtending axillary buds; hence, removal of the apical bud stimulates axillary bud growth (Reece et al., 1946).

As in many herbaceous species, induction of floral morphogenesis in mango is mediated by a stimulus originating in mature leaves and translocated in the phloem (Chacko, 1991; Kulkarni, 1988; Reece et al., 1949). The nature of such stimulus is presently unknown. Photoperiod does not appear to play a direct role in mango flowering (Chacko, 1986). The course of bud morphogenesis in mango is strongly influenced by temperature, presumably through its regulatory role in the activity of the floral stimulus at the time of bud meristematic cell division (Reece et al., 1949).

In this study, the type of morphogenesis (vegetative or floral) exhibited by buds after exposure of trees to water stress was assessed in the summer under warm temperatures, and in the autumn under cool temperatures. Our objective was to determine if water stress induces floral morphogenesis of mango regardless of temperature.

Materials and Methods

Plant Material

Trees used in this study were propagated by air-layering ('Tommy Atkins') or grafting ('Tommy Atkins', 'Keitt', 'Van Dyke', and 'Haden'). 'Tommy Atkins' air-layers were produced from commercial, 10-yr-old trees as previously described (Chapter 3). Branches (2-3 cm in diameter) were girdled by removing a 5-cm ring of bark. The distal edge of the girdle was treated with 2.0% (w/w) NAA in lanolin to induce adventitious rooting. Abundant rooting was observed within 12 weeks of treatment. Air-layers were placed in 12-liter plastic containers in a peat moss and vermiculite medium (1:1 v/v) containing 60 g of Osmocote (14N:14P:14K) and micronutrients. The medium surface was mulched with a 2-cm-thick layer of sphagnum moss to reduce the rate of water loss from the soil surface. Tree canopies were pruned to leave a framework consisting of one or two main branches. Trees were approximately 50 cm tall, with two to four mature (6- to 8-wk-old) vegetative shoots. Shoots emerged from dormant, lateral buds and were nonextended (i.e., they consisted only of the original flush of growth) at the time of initiating treatments. They had dark-green, fully expanded leaves.

Grafted trees of four cultivars were 3-years-old on 'Turpentine' rootstock. These trees were growing in 12-liter containers in a vermiculite:peat moss:silica sand (1:1:1 by volume) medium. Canopies comparable to those of air-layers were generated by pruning as described above.

Assessment of Vegetative Growth and Flowering Morphogenesis

Shoot apices contained visually dormant apical buds (as evidenced by a lack of swelling and tightly clasped external bud scales) at the time of initiating water stress treatments. Dormant buds were, therefore, considered nondifferentiated at the beginning of the experiments, since no primordia had yet been initiated. This feature of mango bud development is in contrast with that of many temperate fruit trees, where buds enter dormancy after forming floral primordia.

Morphogenesis of mango buds that were formed in response to experimental treatments was visually identified upon budbreak by inspecting the axils of external scales. Vegetative buds displayed embryonic leaves with arrested axils, whereas floral buds displayed floral primordia beneath the external scales (Lanuza, 1937; Singh, 1960).

Irrigation and Determination of Plant Water Status

The experiments were conducted under a translucent fiberglass shelter (shade house) or in a glasshouse, to avoid rain and to reduce evaporative demand of trees. No

irrigation was applied during the dry periods to cause water stress. Irrigation consisted of manually applying about 4 liters of water to each control or each water-stressed tree, upon rewatering. This volume of water was sufficient to saturate the medium. Irrigation intervals are specified below for each experiment.

A single leaf was excised from the distal 15 cm of selected vegetative shoots of each tree for determination of LWP. LWP was measured between 0800 and 0930 h using a pressure chamber (Scholander et al., 1965). The intervals for LWP measurement are specified below for each experiment.

Summer Water Stress

'Tommy Atkins' air-layers were water-stressed outdoors during the summer. Trees were irrigated every three to four days prior to the study. The treatments were: 1) control trees irrigated to soil saturation every four days, 2) irrigation suspended for 25 days, and 3) irrigation suspended for 36 days. There were 8 single-tree replicates per treatment in a randomized complete block design. Trees of treatments 2 and 3 were irrigated every four days after the dry period. LWP was measured 0, 14, 25, 36, and 40 days after starting treatments. Minimum ambient temperatures during the experiment were about 20°C. Temperatures occurring during this experiment typically inhibit floral induction in mango. Mango trees in Florida grow

vegetatively during the warm, rainy summer, and do not initiate flowers until cool weather sets in during late autumn and winter.

Autumn Water Stress in Shade House

Tommy Atkins' trees similar to those of the previous experiment were subjected to water stress outdoors starting 11 October 1991. Trees of this experiment were also irrigated about every four days prior to the experiment. Two treatments were tested: 1) control trees irrigated every four days, and 2) irrigation suspended for 35 days, followed by irrigation every four days. There were 8 single-tree replicates per treatment in a randomized complete block design. LWP was measured 0 and 35 days after starting treatments. Cool night temperatures below 15°C, which induce flowering of mango, were occurring during the experimental period.

Autumn Water Stress in Glasshouse

This experiment was initiated 3 November 1991 using grafted trees of 'Tommy Atkins', 'Van Dyke', 'Keitt', and 'Haden' mango. The experiment was conducted in a glasshouse under warm conditions to prevent floral induction in response to cool, outdoor temperatures. Mature vegetative shoots were decapitated at the start of treatment to release axillary bud dormancy. Decapitation consisted of clipping off the apical bud and whorl of distal leaves. Shoots were

decapitated because trees had been growing outdoors, exposed to cool night temperatures which may have pre-conditioned apical buds for floral induction. Axillary buds were dormant and not swollen. Two treatments were applied to trees of the four mango cultivars: 1) control trees irrigated every two days, and 2) irrigation suspended for 14 days. LWP was measured 0 and 14 days after starting treatments. There were 4 single-tree replicates per cultivar per treatment in a randomized complete block design.

Results

Summer Water Stress

Irrigating trees every 4 days during the summer maintained morning LWP above -0.33 MPa (Table 9-1). Leaf-water potentials declined to nearly -2.90 MPa, and below -3.50 MPa, after withholding irrigation for 14 and 25 days, respectively. Withholding irrigation for 36 days caused a further decline in leaf-water potential to -3.78 MPa, and caused defoliation of 3 to 5 leaves per tree in nearly 50% of trees.

All apical buds displayed vegetative morphogenesis (shoot extension), but water stress delayed bud growth (Table 9-2). Twenty-five days after beginning treatments, only 34.8% and 47.6% of apical buds in water-stressed trees were swollen, while 73.7% of apical buds in control trees

Table 9-1. Effect of summer water stress on leaf water potentials of container-grown 'Tommy Atkins' mango trees.

Treatment[a]	Leaf water potential (MPa)[b]				
	Days				
	0	14	25	36	40
Control[c]	-0.32±0.03	-0.29±0.03	-0.28±0.02	-0.31±0.03	-0.28±0.03
25 d dry[d]	-0.33±0.04	-2.88±0.24	-3.55±0.29[e]	-0.22±0.01	-0.28±0.02
36 d dry[d]	-0.30±0.03	-2.76±0.29	-3.61±0.32	-3.78±0.15[e]	-0.52±0.04

[a] Each treatment was applied to eight trees starting 2 July 1991 (day 0).
[b] Values are means ± SE of 8 single-leaf replicates.
[c] Control trees were irrigated every four days.
[d] Irrigation was applied every four days after the dry period.
[e] Determined immediately prior to rewatering.

Table 9-2. Effect of summer water stress on bud growth and morphogenesis in container-grown 'Tommy Atkins' mango.

Treatment[a]	Percent of apical buds initiating growth[b]			
	Days			
	0	25	40	54
Control[c]	0 ± 0.0	73.7 ± 1.1	100.0 ± 0.0	100.0 ± 0.0
25 d dry[d]	0 ± 0.0	47.6 ± 1.2	76.2 ± 0.9	100.0 ± 0.0
36 d dry[d]	0 ± 0.0	34.8 ± 1.8	43.5 ± 1.2	100.0 ± 0.0

[a] Each treatment was applied to eight trees on 2 July 1991 (day 0). The numbers of apical buds per treatment were 19, 21, and 23, for control, 25 d dry, and 36 d dry, respectively.
[b] Apical buds in all treatments exhibited vegetative morphogenesis exclusively. Values are means ± SE.
[c] Control trees were irrigated every four days.
[d] Irrigation was applied every four days after the dry period.

were breaking and displayed emergence of small, tender foliage. Forty days after beginning treatments, all apical buds of control trees had produced extension growth, whereas only 76.2% and 43.5% of apical buds had extended in trees deprived of irrigation for 25 and 36 days, respectively. All apical buds had produced vegetative extension growth on day 54. No flowering occurred for trees in any treatment.

Autumn Water Stress in Shade House

Withholding irrigation for 35 days in October to November resulted in an average leaf water potential of -3.1 MPa (Table 9-3). In contrast to the previous experiment, all apical buds in both water-stressed and well-watered trees initiated flowers. Sixty-two days after beginning the experiment, only 9.5% of apical buds had initiated growth in control trees, compared to nearly 50% in water-stressed trees. The rest of the buds were still dormant and therefore had not initiated growth. All apical buds in trees of both treatments exhibited floral morphogenesis exclusively. On day 62, nearly 50% of apical buds in water-stressed trees had attained budbreak and of these, nearly one-half had developed into panicles measuring 8 to 10 cm in length. In comparison, less than 10% of buds in the control trees had attained budbreak, while the rest of buds were still swollen or dormant. From the difference in panicle development, it was deduced that floral budbreak in water-

Table 9-3. Effect of autumn water stress on leaf water potential, and bud growth and morphogenesis in containerized 'Tommy Atkins' mango.

Trt.[a]	Leaf water potential (MPa)[b]		Percent of apical buds initiating growth[c]		
	Days		Days		
	0	35	35	62	90
Control[d]	-0.32 ± 0.02	-0.24 ± 0.02	0 ± 0.0	9.5 ± 0.2	100 ± 0.0
36 d dry[e]	-0.28 ± 0.02	-3.10 ± 0.31	0 ± 0.0	48.3 ± 0.7	100 ± 0.0

[a] Each treatment was applied to eight trees on 11 October 1991 (day 0).
[b] Values of leaf water potential are means ± SE of 8 single-leaf replicates.
[c] All apical buds in this experiment displayed floral morphogenesis. Values are means ± SE.
[d] Control trees were irrigated every four days (n = 26 apical buds).
[e] Irrigation in the stress treatment was suspended for 35 days, followed by irrigation every four days (n = 29 apical buds).

stressed trees occurred 12-15 days earlier than in control trees. All apical buds of control trees eventually initiated flowers, indicating that water stress was not essential for induction of floral morphogenesis.

Autumn Water Stress in Glasshouse

Trees of this experiment dehydrated rapidly due to the presence of sand in the soil mix and absence of sphagnum moss mulch. However, results of this experiment (Table 9-4) were similar to those obtained in summer, where buds only displayed vegetative morphogenesis (Table 9-2). Water stress during November under warm, glasshouse conditions did not induce flowering in containerized 'Haden' 'Tommy Atkins', 'Keitt', or 'Van Dyke' mango trees. Values of leaf water potential were similar among cultivars. Therefore, data from all four cultivars were pooled. Water-stressed trees attained an average leaf water potential of -3.94 MPa on day 14, compared with about -3.00 MPa for trees of the summer experiment (Table 9-1).

Shoot decapitation released axillary buds from dormancy, and resulted in rapid initiation of growth despite transient exposure to water stress. Twenty days after the experiment was initiated, axillary buds were growing on most decapitated shoots of both control and water-stressed trees (63 and 59% of shoots, respectively). Growing axillary buds were observed on all decapitated shoots on day 45 after

Table 9-4. Effect of autumn water stress on leaf water potential, and bud growth and morphogenesis in grafted 'Tommy Atkins', 'Van Dyke', 'Keitt', and 'Haden' mango. Trees were growing in a temperature-controlled glasshouse at 27 ± 2°C day/22 ± 2°C night, RH > 90%. Vegetative shoots were decapitated to initiate growth of axillary buds.

Trt.[a]	Leaf water potential (MPa)[b]		Percent of decapitated shoots initiating axillary growth[c]		
	Days		Days		
	0	14	14	20	45
Control[d]	-0.39 ± 0.02	-0.41 ± 0.02	0 ± 0.0	63 ± 0.6	100 ± 0.0
14 d dry[e]	-0.36 ± 0.02	-3.94 ± 0.23	0 ± 0.0	59 ± 0.7	100 ± 0.0

[a] Treatments were applied 3 November 1991 (day 0). Data from all four cultivars (4 trees per cultivar per treatment) were pooled, therefore, each treatment consisted of 16 trees.
[b] Values of leaf water potential are means ± SE of 16 single-leaf replicates.
[c] All axillary buds exhibited vegetative morphogenesis exclusively.
[d] Control trees were irrigated every two days (n = 57 shoots).
[e] The water stress treatment consisted of suspending irrigation for 14 days, followed by irrigation every two days (n = 49 shoots).

starting the experiment. All growing buds displayed vegetative morphogenesis exclusively.

Discussion

Water stress applied during warm or cool temperatures did not induce floral morphogenesis in dormant buds of container-grown mango trees in this study. However, water stress applied during cool weather in the autumn caused earlier floral budbreak.

Irrigation or water stress in the summer resulted in vegetative morphogenesis exclusively. In studies with other mango cultivars, water stress also failed to induce flowering. In South Africa, container-grown 'Haden' and 'Sensation' mango trees were water-stressed for eight weeks beginning in late March (the normal flowering period is July-August). Unfortunately, LWP was not measured in that study to assess plant water status. Buds of both water-stressed and irrigated trees exhibited only vegetative morphogenesis (Wolstenholme and Hofmeyr, 1985). In 'Tommy Atkins' mango, water stress delayed vegetative growth. Nearly twice as many apical buds of irrigated trees had attained budbreak compared to water-stressed trees (Table 9-2). Bud growth was likely repressed by a temporary decrease in cell turgor during plant water stress.

Applying water stress in October, when night temperatures were about 15°C, did not increase the total

amount of apical buds displaying floral morphogenesis; all buds of stressed and control trees produced flowers (Table 9-3). In low temperatures, floral buds were initiated regardless of water stress, although water stress advanced floral budbreak of nearly 40% of buds by about two weeks. Buds did not show visual signs of growth during the period of water stress. At that time, dehydration may have caused physiological alterations, perhaps hormonal, that resulted in enhanced bud activity upon rewatering.

We observed that water-stressing trees of four different mango cultivars during autumn in a warm glasshouse, did not induce flowering, since dormant axillary buds on decapitated shoots produced new vegetative growth exclusively (Table 9-4). In contrast, irrigated trees (n = 6) of these cultivars that remained outdoors exposed to low temperatures produced 2 to 4 axillary floral buds per decapitated shoot. Although dormant axillary buds exhibited morphogenetic responses to temperature similar to those of dormant apical buds, growth of axillary buds initiated more rapidly due to the dormancy-releasing effect of shoot decapitation.

The effects of water stress and temperature on containerized mango appear similar to those reported for lychee. As in mango, lychee vegetative shoots produce apical panicles after a period of dormancy. When container-

grown lychee trees were water-stressed for seven weeks to a water potential of -1.50 MPa, and rewatered to initiate growth at 15°C day/10°C night, floral buds were formed (Menzel and Simpson, 1990). However, only vegetative buds were formed when trees were water-stressed, and rewatered to initiate growth at 30°C day/25°C. As in the present study with mango (Table 9-2), vegetative flushing of lychee was delayed by water stress. It was proposed for lychee that the role of water stress in flowering was indirect, and consisted in repressing vegetative growth during warm weather until temperatures below 20°C in the autumn-winter induced floral morphogenesis and floral initiation ensued (Menzel and Simpson, 1990). Results of our study suggest that a similar phenomenon occurs in mango. We observed that water stress delayed growth of vegetative buds (shoot extension) under warm conditions. We also noted, however, that water stress accelerated growth of floral buds that had been induced by low temperatures. In areas with mild winters, water stress before the flowering period may thus prevent shoot extension, increasing the number of dormant buds available to receive the leaf-generated signal for floral induction. Water stress under those conditions may also extend the cropping season by advancing the flowering period.

The mechanism of mango floral induction in warm environments remains elusive. Young mango leaves appear incapable of producing floral stimulus, since flowers are initiated on dormant vegetative shoots whose leaves are fully expanded, dark green, and lignified. Water stress restricts production of new leaves in mango and can, consequently, increase the proportion of inductive leaves relative to young ones. It is therefore possible that under marginally-inductive temperatures in the tropics, mango floral induction can occur after a period of plant water stress, when canopies consist mainly of mature, inductive leaves.

CHAPTER 10
FLOWERING RESPONSES TO EXOGENOUS GIBBERELLIN

GA_3 antagonizes flowering in many woody perennials (Pharis and King, 1985), including mango (Kachru et al., 1971; Tomer, 1984; Andrews and Le Fook, 1985; Galán-Saúco, 1990). Flowering of 'Keitt', 'Tommy Atkins', 'Haden', and 'Palmer' mango trees was prevented by sprays of 25 to 200 $mg \cdot l^{-1}$ GA_3 (Tomer, 1984). Kachru et al. (1971) reported that 10^{-1} M and 10^{-2} M GA_3 prevented floral differentiation in 'Dashehari' mango, whereas 10^{-3} M and 10^{-4} M GA_3 delayed floral bud break by nearly two weeks when compared to nontreated shoots.

The effects of GA_3 sprays on vegetative growth of mango are unclear. Dormant buds of *Citrus*, in common with those of mango, are nondifferentiated (Lord and Eckard, 1987). GA_3 prevented initiation of floral primordia in *Citrus*, but the initiation of vegetative primordia was not affected by GA_3 (Guardiola et al., 1977, 1982). In *Citrus latifolia*, GA_3 caused buds to differentiate as vegetative shoots during a period in which they normally form inflorescences (Davenport, 1983).

Temperature exerts a strong influence in bud morphogenesis of mango. Cool temperatures of about 15°C promote flowering, whereas temperatures near 30°C tend to promote vegetative growth instead of flowering (Shu and Sheen, 1987; Whiley, et al., 1989). Floral induction involves a floral stimulus originating in mature leaves (Reece et al. 1946, 1949; Singh, 1959; Kulkarni, 1986, 1988). Dormant buds of mango are nondifferentiated, containing an arrested apical meristem and preformed nodes, but no differentiated vegetative or floral primordia (Lanuza, 1937; Mustard and Lynch, 1946; Singh, 1958; Scholefield et al., 1986). Upon initiating floral or vegetative morphogenesis, bud differentiation is continuous and rapid. Inflorescences usually reach anthesis within eight weeks after the start of bud differentiation. Axillary buds, which are inhibited by the presence of the apical bud, can be released from dormancy and stimulated to undergo growth and differentiation by decapitation of shoots (Reece et al., 1946).

The present work describes the effects of GA_3 sprays on 'Tommy Atkins' and 'Keitt' mango trees when applied under cool, floral-promotive, or warm temperatures which typically promote vegetative growth rather than flowering. The objective was to determine the role of GA_3 in bud dormancy

and morphogenesis in these two contrasting temperature conditions.

Materials and Methods

Plant Material, Temperature Conditions, and GA_3 Sprays

Plants of 'Keitt' and 'Tommy Atkins' mango, consisting of adult, 15-yr-old orchard trees and air-layers in 12-l containers (Chapter 3) were used in this study. Orchard trees were sprayed with GA_3 during the winter (about 26°C day/15°C or lower at night) or summer (about 29°C day/25°C night). Individual branches bearing several flowering shoots (i.e., shoots bearing an apical inflorescence), or vegetative shoots, were selected for treatments during the winter and summer, respectively.

Air-layers were grown outdoors during a period of warm temperatures (day/night temperatures above 27°/22°C) prior to treatment. Canopies contained 3 to 6 vegetative shoots, each with 2-3 growth flushes. The terminal vegetative flush in all shoots was between 7 and 9 weeks old at the time of treatment.

Air-layers were placed inside growth chambers under cool (18°C day/10°C night, 12-h photoperiod) or warm (30°C day/25°C night, 12-h photoperiod) temperature regimes. Photosynthetic photon flux in the growth chambers was about 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the upper canopy level. Trees were

irrigated to soil saturation on alternate days to prevent possibly confounding effects of water stress.

GA_3 spray solutions of 0 to 250 $mg.l^{-1}$ in water included 0.1 % (V/V) of the surfactant, Triton X-100. All treatments consisted of a single spray applied to runoff on all deblossomed or clipped shoots. All experiments were set up in a randomized complete block design.

Release of Dormant Axillary Buds

Axillary buds on vegetative shoots were released from dormancy by clipping the terminal flush of growth, immediately above the constriction separating the terminal and subterminal flushes. Terminal flushes contained fully-expanded, dark green leaves at the time they were detached. In flowering shoots, axillary buds were released by cutting the apical inflorescence at its base (deblossoming). Inflorescences were at or near anthesis when removed from the shoots. Clipping and deblossoming stimulated growth of the dormant axillary buds, but did not determine their floral or vegetative fate. For containerized trees, released axillary buds of all clipped shoots were allowed to undergo growth and differentiation in the corresponding temperature regime prior to removing trees from the growth chambers.

Effect of GA_3 Sprays on Orchard Trees During the Flowering Period (Cool Weather)

Flowering shoots of orchard, 8-yr-old, 'Keitt' and 'Tommy Atkins' mango trees were sprayed with GA_3 in late December to determine its effect on growth and morphogenesis of axillary buds. Five trees of each cultivar were used, and four branches were selected per tree. Each branch had 12 to 20 flowering shoots which were deblossomed and sprayed with 0, 10, 50, or 250 $mg \cdot l^{-1}$ GA_3. Deblossomed shoots were observed on five occasions over a 13-week period (from mid-January to mid-April) following treatment. Average day/night temperatures were below 26°C/15°C from mid-January to late March, but they generally exceeded 28°/18°C afterwards.

Effect of GA_3 Sprays on Containerized Trees During Exposure to a Cool Temperature Regime in the Growth Chamber

Terminal flushes of vegetative shoots were clipped off during mid-May to release dormant buds. At this time, tree canopies were sprayed with 0 or 250 $mg \cdot l^{-1}$ GA_3. Sprayed trees (n = 15) were transferred to the cool growth chamber to allow buds to undergo growth and differentiation. Trees were transferred back to the warm, outdoor conditions after axillary buds attained bud break. At this time, buds were at least 2 cm long and one could distinguish with ease whether they were developing as inflorescences or vegetative shoots.

Effect of GA_3 Sprays on Orchard Trees During the Period of Vegetative Growth (Warm Weather)

Five 10-yr-old orchard trees each of 'Keitt' and 'Tommy Atkins' were used in this experiment to determine the effect of GA_3 sprays during warm summer months, the period of vegetative growth of mango trees in south Florida. In late July, three branches comprising 12 to 22 vegetative shoots were selected per tree. The terminal vegetative flush of each shoot was clipped to stimulate growth and differentiation of axillary buds. All clipped shoots within each branch were sprayed with 0, 50 or 250 mg.l-1 GA_3.

Effect of GA_3 Sprays on Containerized Trees During Exposure to a Warm Temperature Regime in the Growth Chamber

During August, terminal vegetative flushes of shoots were removed in containerized 'Keitt' and 'Tommy Atkins' mango trees to stimulate growth and differentiation of axillary buds. Two sets of trees (n = 15) were each sprayed with 250 mg.l-1 GA_3 plus 0.1% (V/V) Triton X-100 or sprayed with the surfactant only.

Results

Effect of GA_3 Sprays on Orchard Trees During the Flowering Period (Cool Weather)

GA_3 quantitatively delayed growth and differentiation of dormant axillary buds in both 'Keitt' (Fig. 10-1) and 'Tommy Atkins' (Fig. 10-2). The greatest retarding effect

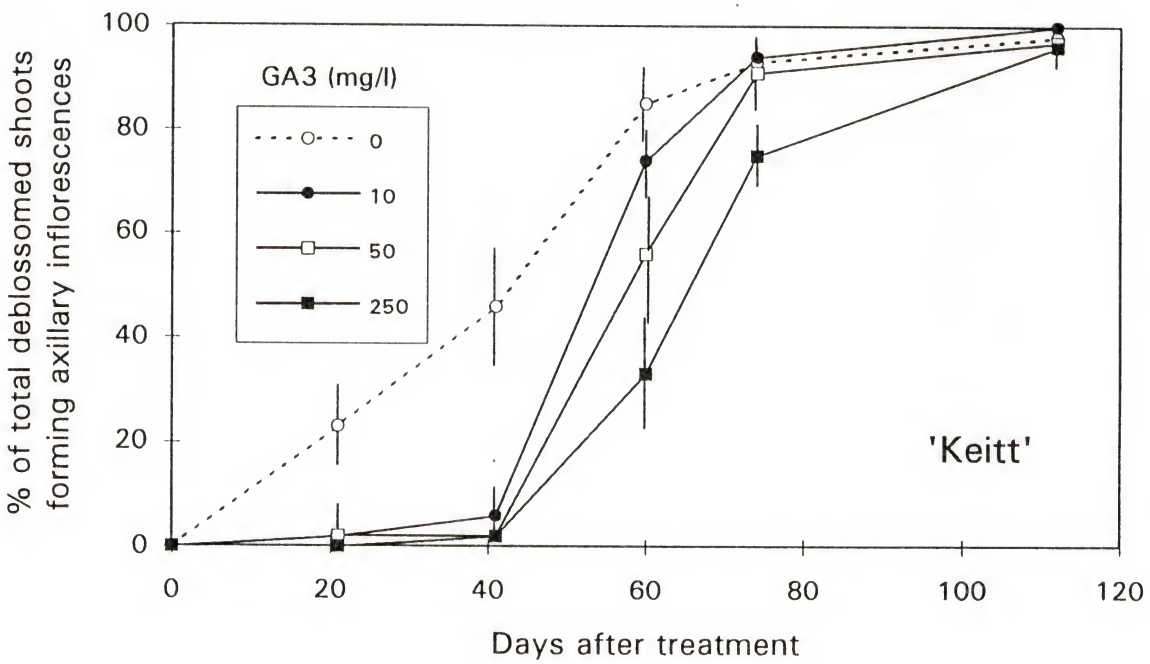

Figure 10-1. Effect of GA_3 on axillary flowering of deblossomed shoots in orchard trees of 'Keitt' mango. Flowering shoots were deblossomed in late December by removing the apical inflorescence. Each data point represents the percent of deblossomed shoots ± SE in which axillary buds differentiated as inflorescences. Each treatment consisted of a single spray applied at the time of deblossoming to four branches (having a total of at least 58 deblossomed shoots) in each of 5 trees.

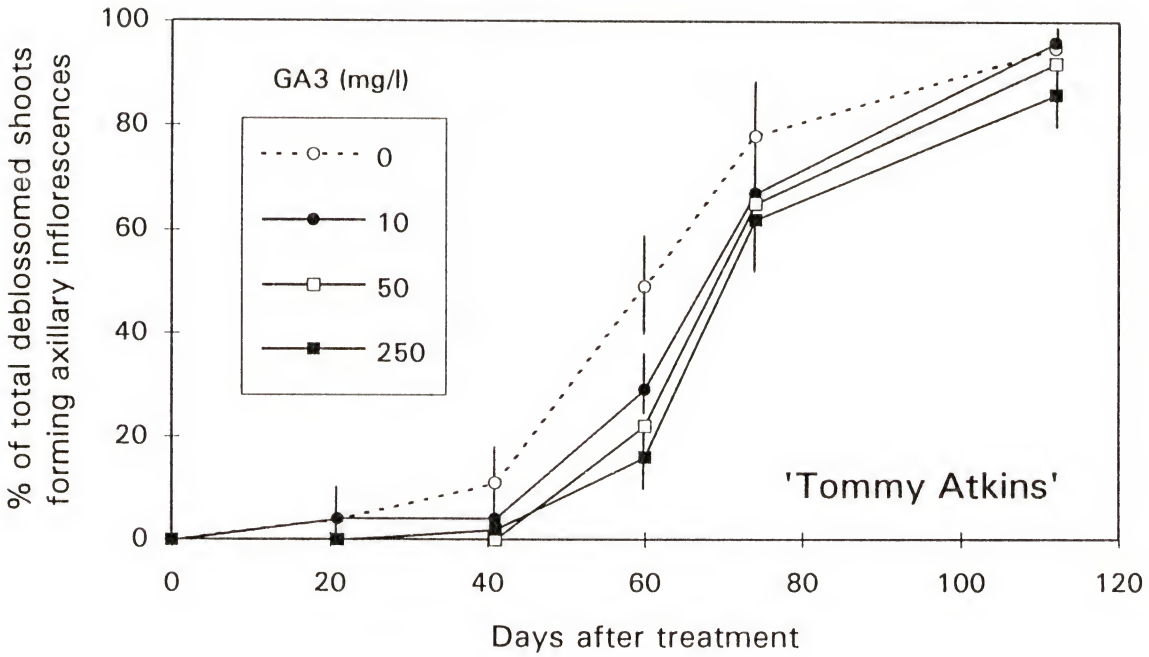

Figure 10-2. Effect of GA_3 sprays on axillary flowering of deblossomed shoots in orchard trees of 'Tommy Atkins' mango. (See Fig. 10-1 for details.)

occurred when the deblossomed shoots were sprayed with 250 $mg \cdot l^{-1}$ GA_3. For example, 60 days after imposing the treatments (25 February), the amount of deblossomed shoots treated with 250 $mg \cdot l^{-1}$ GA_3 that displayed actively growing axillary buds was about one-half of the nonsprayed, control shoots in both cultivars. All released axillary buds developed into inflorescences. By 74 days after starting the experiment (11 March), most deblossomed, GA_3-sprayed shoots of both cultivars displayed axillary inflorescences. On day 112 (18 April), nearly all deblossomed shoots of both cultivars, including those sprayed with 250 $mg \cdot l^{-1}$ GA_3, displayed axillary inflorescences; however, most inflorescences developing on the latter shoots were clearly less developed than those produced in shoots of the other treatments. Whereas more than 90% of deblossomed shoots had produced axillary inflorescences by day 112, only 2% to 8% of the deblossomed shoots had produced axillary vegetative shoots.

Effect of GA_3 Sprays on Containerized Trees During Exposure to a Cool Temperature Regime in the Growth Chamber

As in the previous experiment, axillary buds of clipped shoots treated with various rates of GA_3 produced mainly inflorescences. In contrast to the previous experiment, however, GA_3 in this experiment did not retard the growth of axillary buds of clipped shoots of 'Keitt' (Fig. 10-3)

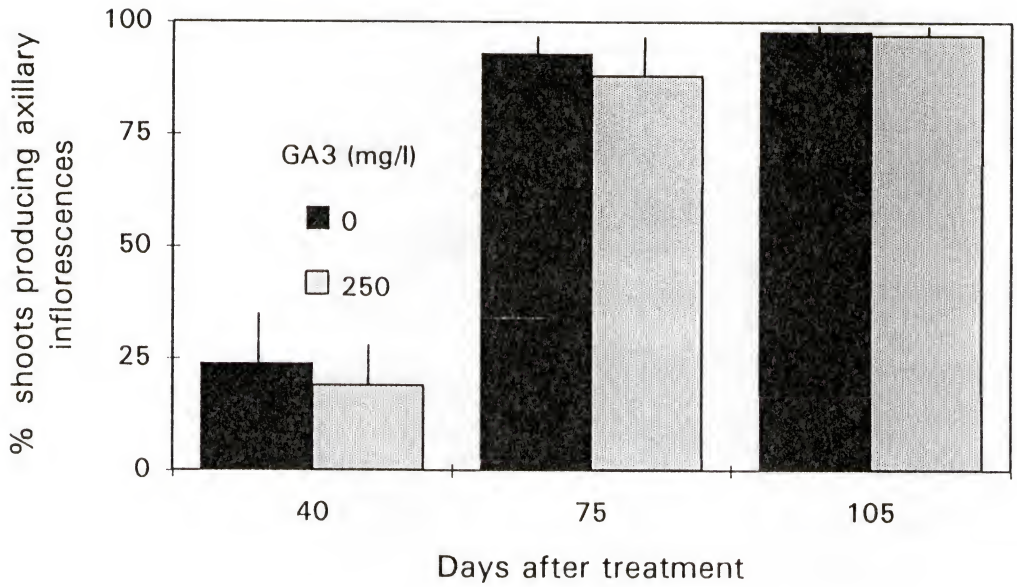

Figure 10-3. Effect of GA_3 sprays on axillary flowering of clipped vegetative shoots in containerized trees of 'Keitt' mango. Vegetative shoots were clipped in mid-May by removing the terminal flush of growth. Trees were placed inside a growth chamber at 18°C day/10°C night, 12-h photoperiod. Each bar represents the percent of clipped shoots ± SE in which axillary buds differentiated as inflorescences. Each treatment consisted of a single spray applied to 15 trees (at least 60 clipped shoots per treatment).

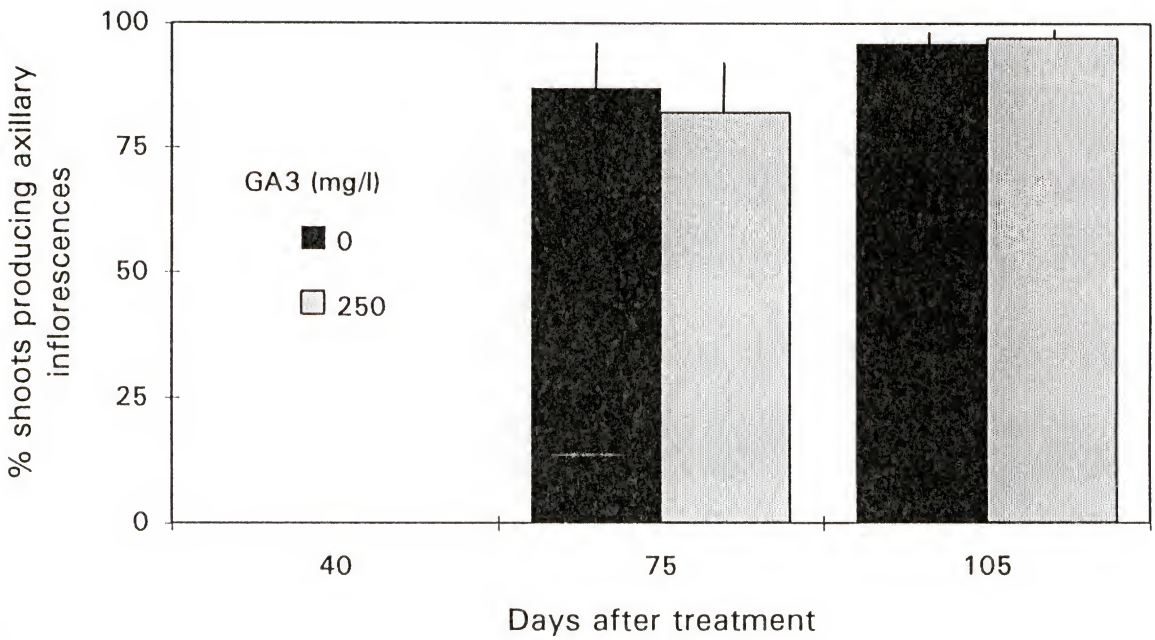

Figure 10-4. Effect of GA_3 sprays on axillary flowering of clipped vegetative shoots in containerized 'Tommy Atkins' mango trees. (See Fig. 10-3 for details.)

or 'Tommy Atkins' trees (Fig. 10-4). Axillary buds of 'Keitt' initiated floral morphogenesis sooner than those of 'Tommy Atkins' trees, but on day 75, the total amounts of clipped shoots producing axillary inflorescences were similar for all treatments. On day 105, axillary buds of virtually all clipped shoots displayed floral development.

Effect of GA_3 Sprays on Orchard Trees During the Period of Vegetative Growth (Warm Weather)

All released axillary buds in this experiment produced new vegetative shoots. GA_3 at 50 or 250 $mg \cdot l^{-1}$ did not delay axillary bud growth in clipped shoots of either 'Keitt' (Fig. 10-5) or 'Tommy Atkins' trees (Fig. 10-6). Axillary buds of all experimental shoots displayed growth within 2 wk of starting the experiment. At least two thirds of shoots of each treatment displayed actively growing buds on day 27. On day 43, nearly all experimental shoots had new axillary vegetative shoots regardless of GA3 application or rate.

Effect of GA_3 Sprays on Containerized Trees During Exposure to a Warm Temperature Regime in the Growth Chamber

As in the previous experiment, all axillary buds released in this experiment produced vegetative shoots (Figs. 7 and 8). A spray of 250 $mg \cdot l^{-1}$ GA_3 applied while trees were exposed to 30°C day/25°C night in the growth chamber did not affect the time at which released axillary

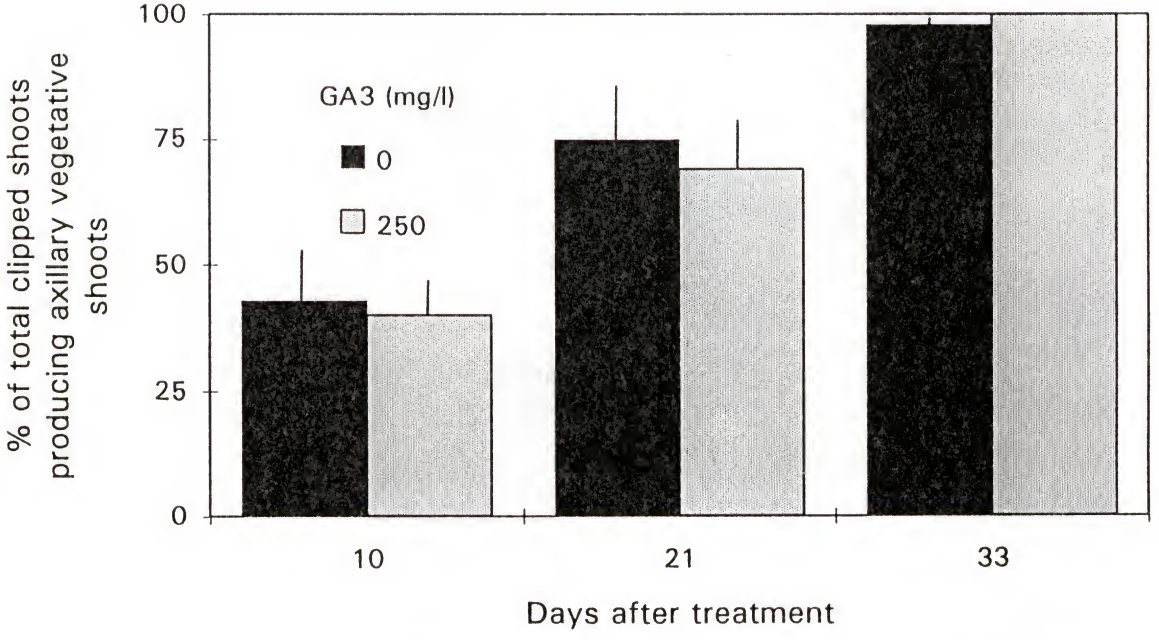

Figure 10-5. Response of orchard trees of 'Keitt' mango to exogenous GA_3 applied during the period of vegetative growth in the summer (temperatures about 29°C day/25°C night). Shoots were clipped (the terminal flush was removed) to stimulate growth of dormant axillary buds. Five trees (three branches per tree; at least 42 shoots) were used per treatment. All released axillary buds produced new vegetative shoots.

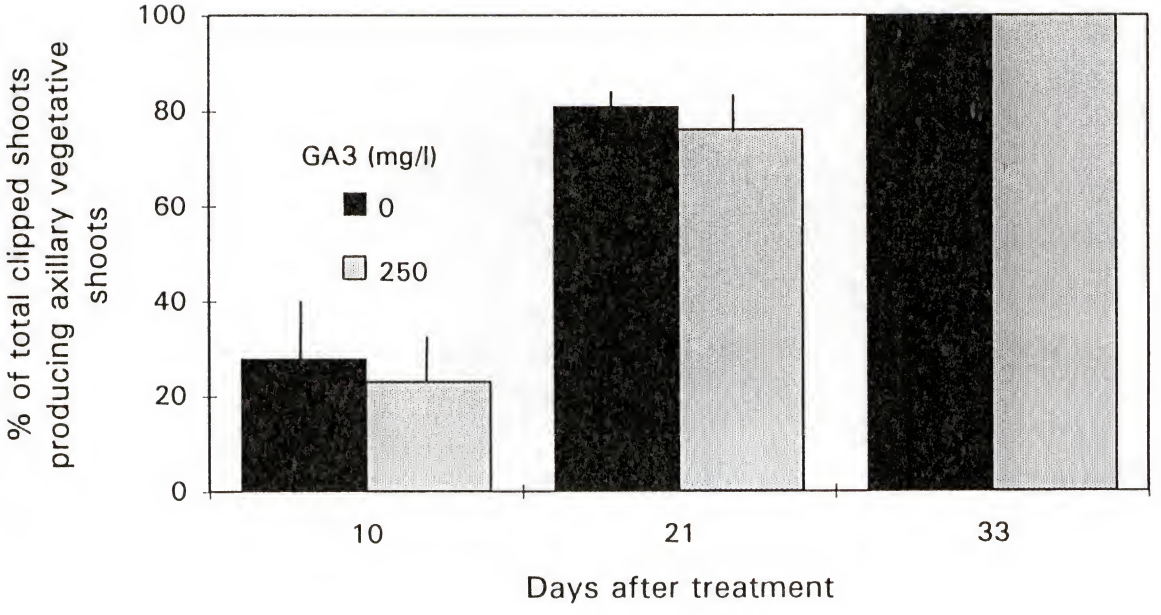

Figure 10-6. Response of orchard trees of 'Tommy Atkins' mango to exogenous GA_3 applied during the summer. (See Fig. 10-5 for details.) All new axillary shoots were vegetative.

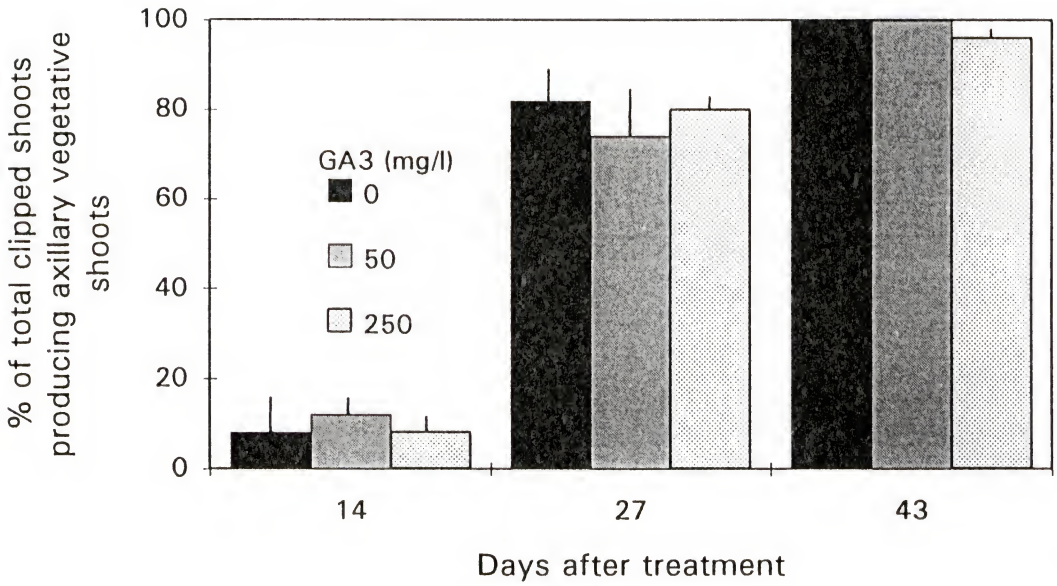

Figure 10-7. Growth responses of 'Keitt' mango trees to exogenous GA_3 applied under warm temperature conditions (30°C day/25°C night, 12-h photoperiod) in a growth chamber. Containerized trees (n = 15) were subjected to a noninductive temperature regime of 30°C day/25°C night, 12-h photoperiod. Vegetative shoots were clipped to stimulate formation of axillary buds.

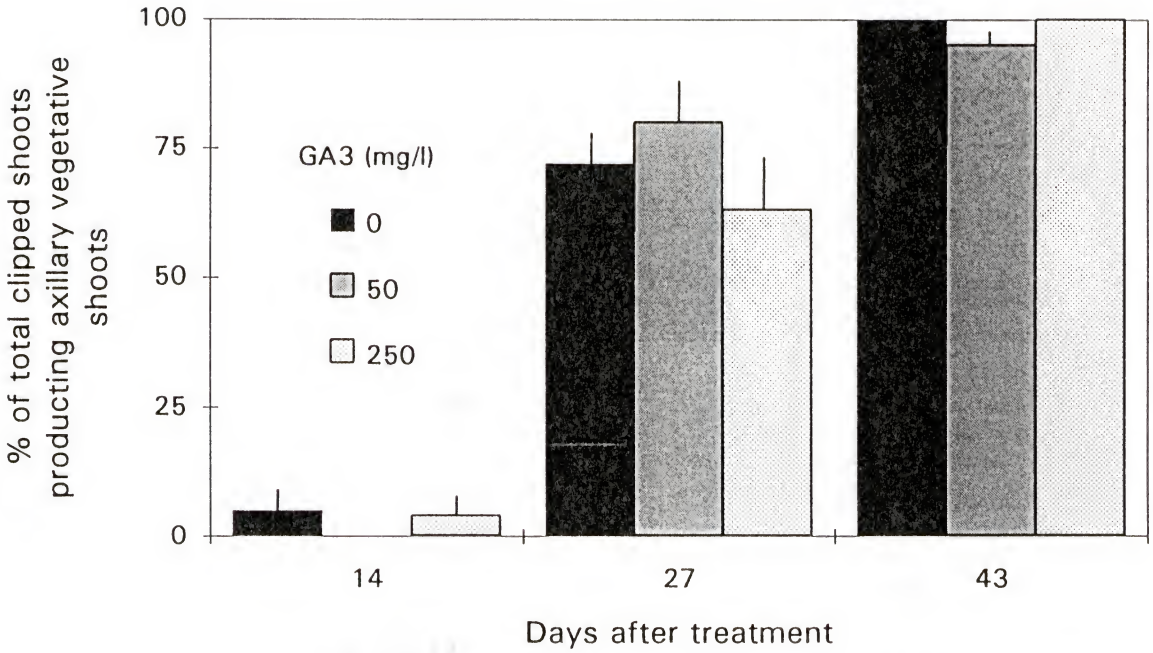

Figure 10-8. Growth responses of 'Tommy Atkins' mango to exogenous GA_3 applied under warm temperature conditions in a growth chamber. (See Fig. 10-7 for details.)

buds commenced vegetative morphogenesis in relation to buds of control shoots. Bud growth commenced within 10 days after starting the experiment. Over time, the proportions of clipped shoots producing axillary vegetative shoots were similar for control and GA_3-treated trees of both cultivars.

Discussion

This study was conducted to determine the effects of exogenous GA_3 on growth (dormancy release) and the type of morphogenesis expressed by axillary buds of mango. GA_3 was sprayed in cool, floral-promotive temperatures, and in warm temperatures which typically stimulate vegetative development rather than flowering. GA_3 delayed growth of axillary buds of deblossomed shoots in both 'Keitt' and 'Tommy Atkins' orchard trees (Figs. 10-1 and 10-2). Axillary buds differentiated as inflorescences in most control and GA_3-sprayed shoots, but there was an increasing delay in floral bud initiation as higher GA_3 doses were applied. Floral initiation occurred presumably because buds underwent growth and differentiation during a period of cool weather in which night temperatures were below 15°C. Such cool temperatures are thought to promote flowering by allowing the synthesis or action of the putative leaf-generated floral stimulus (Reece et al., 1949; Kulkarni, 1986, 1988). In a few deblossomed shoots (only 8% or less) axillary buds developed into new vegetative shoots. This

response, however, was observed only in GA_3-sprayed shoots whose axillary buds underwent delayed growth and differentiation until mid-April, when average day/night temperatures were above 25°C/18°C. GA_3 did not cause buds to differentiate vegetatively when growth occurred during cool temperatures. Thus, the effect of GA_3 was to delay bud growth and differentiation during cool temperatures, whereas warm temperatures near 30°C inhibited the initiation of floral primordia regardless of GA_3. In other studies where GA_3 application has resulted in vegetative growth rather than flowering (e.g., Kachru et al., 1971), bud growth and differentiation also occurred towards the end of the flowering period, and it is possible that, at that time, cool temperatures had receded. In the study of Tomer (1984), GA_3 prevented apical buds of 'Tommy Atkins' and 'Keitt' mango from initiating floral morphogenesis throughout the entire flowering period in the winter. Unfortunately, the subsequent developmental fate of the repressed apical buds during warm weather in the spring-summer was not reported. It seems reasonable to assume, however, that such buds produced a vegetative flush after cool temperatures receded and the exogenous GA_3 was metabolized.

In the second experiment (Figs. 10-3 and 10-4), clipped vegetative shoots of trees growing in containers were

sprayed with 0 or 250 $mg \cdot l^{-1}$ GA_3 to determine whether GA_3, when applied in the presence of cool temperatures, would cause axillary buds to differentiate as vegetative shoots instead of inflorescences. In all clipped shoots, axillary buds formed only inflorescences. In this experiment, however, floral initiation was not delayed by GA_3 as in the previous experiment with deblossomed shoots (Figs. 10-1 and 10-2). Such contrasting responses to GA_3 suggest that the recent developmental history of shoots influenced the sensitivity of dormant buds to applied GA_3. When GA_3 was applied on deblossomed shoots of orchard trees during the winter, it delayed floral morphogenesis in axillary buds (Figs. 10-1 and 10-2). Those experimental shoots had already flowered as a result of exposure to low night temperatures around 15°C for more than five weeks. It is possible, therefore, that axillary buds had already attained floral induction during exposure to low temperatures, but remained dormant owing to the presence of the apical inflorescence (Reece et al., 1946, 1949). After shoots were deblossomed, GA_3 prolonged dormancy of the florally-induced buds. In contrast, the presumably induced axillary buds of nonsprayed shoots quickly commenced growth and floral differentiation after deblossoming. The present results indicate that GA_3 prolonged dormancy of induced buds, whereas growth and differentiation of noninduced buds (buds

that developed vegetatively) was not retarded. The basis for the selective retarding effect of GA_3 on dormant buds that are not yet differentiated but apparently have reached floral induction is unclear. In *Citrus*, buds that eventually formed purely generative inflorescences were more sensitive to the growth-inhibiting effect of GA_3 than buds forming leafy inflorescences or vegetative shoots (Guardiola et al., 1982; Guardiola et al., 1977).

GA_3 at up to 250 $mg \cdot l^{-1}$ did not affect the release of bud dormancy in either 'Keitt' or 'Tommy Atkins' trees when applied during warm conditions outdoors (Figs. 10-5 and 10-6) or in the growth chamber (Figs. 10-7 and 10-8). The lack of effect of GA_3 on vegetative differentiation of buds further supports the suggestion that the response of axillary buds to applied GA_3 was influenced by the recent vegetative or floral activity of shoots. The delaying effect of GA_3 on floral initiation was observed in axillary buds of deblossomed shoots (buds that presumably had reached floral induction after exposure to low temperatures), but not in axillary buds of clipped vegetative shoots (buds which had not attained floral induction). In *Citrus*, gibberellin levels above a certain threshold are suggested to inhibit initiation of floral primordia after floral induction has taken place (Goldschmidt and Monselise, 1972; Guardiola, 1981; Lord and Eckard, 1987).

In the present experiments, GA_3 had no effect on bud dormancy or morphogenesis when treated buds underwent differentiation in warm temperatures. GA_3 did not cause vegetative morphogenesis when treated buds underwent differentiation in cool temperatures, but it delayed floral initiation. Thus, the role of GA_3 in flowering of mango does not seem to consist of preventing floral induction. Rather, GA_3 appears to delay the initiation of floral primordia (prolong dormancy) in dormant buds that have already attained floral induction.

CHAPTER 11
FLOWERING RESPONSES TO GIBBERELLIN-BIOSYNTHESIS INHIBITORS

The control of vegetative and reproductive development in tropical evergreen trees, including mango, is complex and poorly understood. Growth of mango occurs by sporadic flushing of sections of the canopy. Vegetative shoots display episodic extension and terminate when an apical reproductive bud is differentiated (Hallé et al., 1978; Singh, 1960). Reproductive buds are purely floral, developing into completely generative panicles, or mixed, forming mixed panicles that bear both leaves and axillary flower clusters.

Floral development in mango is not interrupted by a period of dormancy as occurs in most temperate fruit trees (Mustard, 1946; Singh, 1960; Scholefield et al., 1986). Mango floral induction is presumably caused by a stimulus originating in mature leaves (Chacko, 1986, 1991; Kulkarni, 1986; Reece et al., 1949). The floral stimulus causes floral differentiation, but it does not appear to regulate meristematic cell division (Reece et al., 1949). Despite the possibility of manipulating expression of floral and

vegetative development in mango, (Reece et al., 1949; Kulkarni, 1986), the nature of the floral stimulus is uncertain (Chacko, 1991).

Mango growth and development are strongly influenced by the environment. Floral induction is promoted by temperatures of about 15°C, whereas vegetative growth is generally promoted by temperatures above 20°C (Shu and Sheen, 1987; Whiley et al., 1989).

The triazoles, paclobutrazol (PBZ) and uniconazole (UCZ), are potent inhibitors of gibberellin biosynthesis which can suppress vegetative growth and cause profuse and early flowering of mango (Burondkar and Gunjate, 1991; Charnvichit and Tangumpai, 1991; Kulkarni, 1988; Rukayah, 1990). To determine whether these retardants could cause floral induction of 'Tommy Atkins' mango, trees were sprayed with PBZ or UCZ, and subjected to a chilling (18/10°C day/night), floral-inductive, or warm (30/25°C day/night), noninductive regime.

Materials and Methods

Trees of 'Tommy Atkins' mango were produced by air-layering (Chapter 3) and potted during the spring in 12-liter containers. A 1:1 (v/v) soil mix of peat moss and vermiculite was used. Trees were defoliated and selectively pruned to generate vegetative shoots of similar age. Trees with 4 to 7 tender, nonextended vegetative shoots ca. 3 wk

old were sprayed with an aqueous suspension of 2,000 $mg.l^{-1}$ PBZ (Burondkar and Gunjate, 1991) or 500 $mg.l^{-1}$ UCZ. Suspensions included 1.0 ml l^{-1} of the surfactant, Triton X-100. All trees were kept outdoors under partial shade until shoots were 6 to 7 wk old and had dark green, fully-expanded leaves. Trees were then subjected to either 18/10°C or 30/25°C day/night regimes in separate growth chambers. Both chambers were programmed for a 12-h photoperiod, 300 to 350 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic photon flux and RH > 75%. Nonsprayed trees served as controls in each chamber. All trees were watered to soil saturation every 3 to 4 days during the study. Each of the six treatments was applied to seven trees.

Shoot apices of all trees were visually dormant at the start of the study. Initiation of apical buds was stimulated at the start of temperature treatment by defoliating the shoot tips (Chapter 6). Bud initiation was characterized as the visual swelling and rising (about 5 mm in height) of the apex, which assumed a distinct conical shape, and had tightly closed outer bud scales. Bud break was considered the stage at which external bud scales loosened and began to open.

All trees were returned outdoors after 75 days of enclosure in the growth chambers. Nearly all shoot apices initiated buds within this period. Budbreak and

morphogenesis were evaluated after 0, 25, 43, 61 and 75 days of enclosing trees in the growth chambers.

Results

Bud Initiation and Budbreak

At 18/10°C both PBZ and UCZ advanced bud break (Fig. 11-1). After 25 days of chilling, 3% of apical buds in PBZ-sprayed shoots had attained bud break, whereas no bud break was yet evident in control and UCZ-sprayed shoots. After 43 days of chilling, 62 and 66% of buds in PBZ- and UCZ-sprayed shoots had attained bud break, whereas only 12% of shoot apices in control trees had breaking buds. After 61 days of chilling, more than 90% of both triazole-sprayed and control shoots had produced apical growth. More than 95% of shoot apices initiated buds within 75 days of chilling in every treatment.

As expected, bud initiation occurred sooner at 30/25°C than at 18/10°C. Warm temperatures caused 32% of shoot apices in control trees to attain bud break after 25 days of treatment, while of the triazole treatments, only UCZ caused bud break (4%) during this time (Fig. 11-2). After 61 days of warm temperature treatment, 89 to 98% of apical buds in all three treatments had attained bud break. All apical buds had attained bud break by day 75.

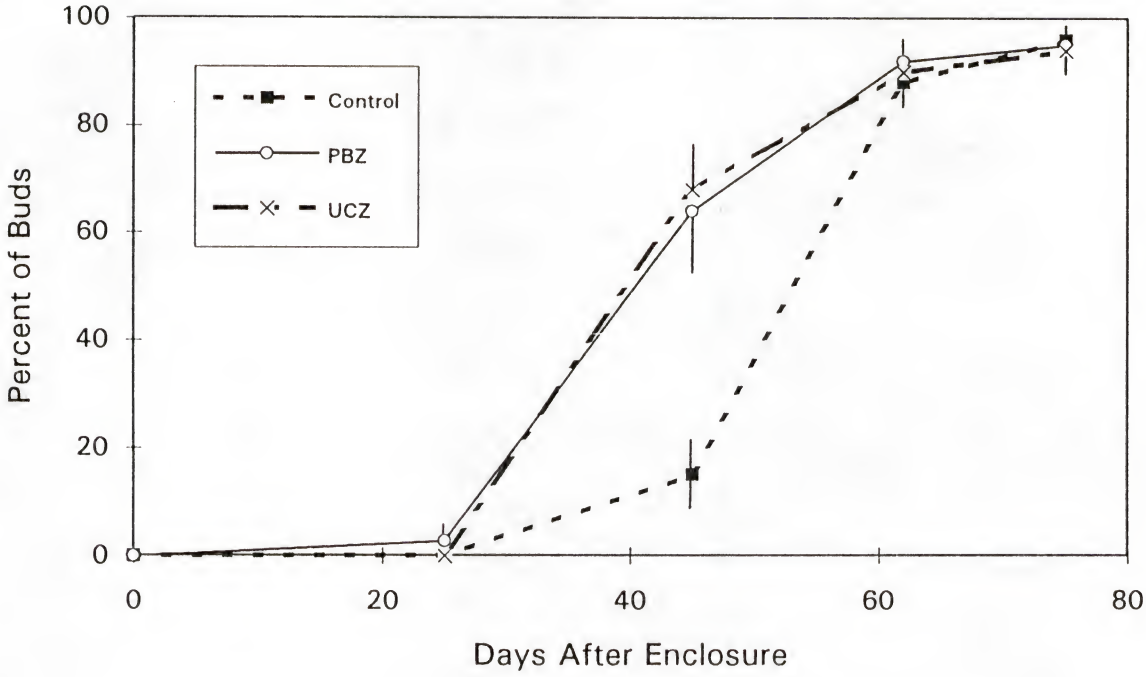

Figure 11-1. Apical bud break in 'Tommy Atkins' mango as influenced by chilling at 18/10°C day/night and a spray of 2,000 $mg.l^{-1}$ paclobutrazol (PBZ) or 500 $mg.l^{-1}$ uniconazole (UCZ). PBZ and UCZ were sprayed on 3-wk-old shoots, and trees were placed in controlled-environment chambers when shoots were 6-7 weeks old. Each symbol represents the cumulative percentage of apical buds within each treatment ($n > 32$) attaining bud break.

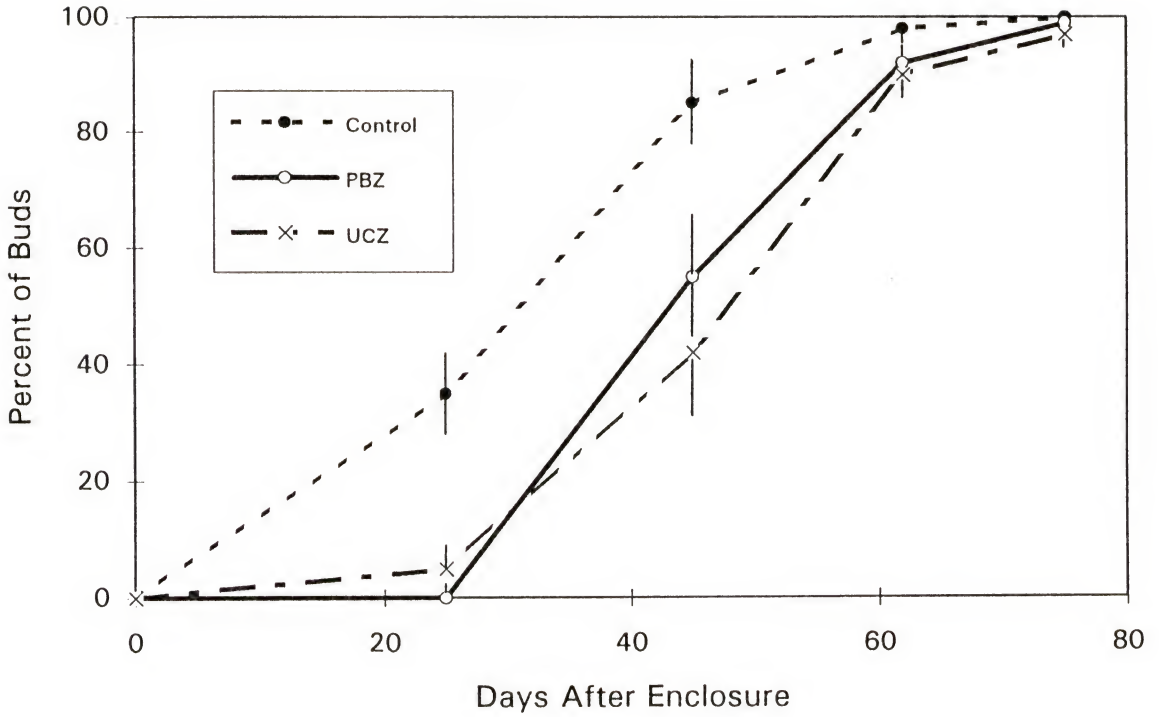

Figure 11-2. Apical bud break in 'Tommy Atkins' mango as influenced by a spray of 2,000 $mg \cdot l^{-1}$ paclobutrazol (PBZ) or 500 $mg \cdot l^{-1}$ uniconazole (UCZ), and subsequent treatment at 30/25°C day/night. PBZ and UCZ were sprayed on 3-wk-old shoots, and trees were placed in controlled-environment chambers when shoots were 6-7 weeks old. Each symbol represents the cumulative percentage of apical buds within each treatment ($n > 31$) attaining bud break.

Bud Morphogenesis

Shoot apices initiated floral, mixed, or vegetative buds during temperature treatment. Most buds that were initiated at 18/10°C were floral regardless of triazole pre-treatment (Fig. 11-3). Nonsprayed shoot apices initiated mainly floral buds (74%), but also mixed buds (11%) and vegetative buds (15%). Both PBZ and UCZ increased production of floral buds (>90%) at 18/10°C in relation to control shoots (74%), and caused moderate compaction of floral and mixed panicles. No reproductive buds were initiated at 30/25°C, where shoot apices only initiated vegetative buds.

Vegetative Flushing

Frequency of vegetative flushing was reduced by both PBZ and UCZ (Fig. 11-4). After 75 days of treatment, all shoots had produced at least one flush, but 82% of control shoots had already flushed twice. In addition to inhibiting vegetative flushing, PBZ and UCZ shortened internode lengths to about 30% that of controls (data not shown).

Discussion

This study provides evidence that bud morphogenesis in mango is determined by temperature. Floral initiation occurred only under the chilling treatment. The results may be explained by the activation of a floral stimulus under chilling conditions (Reece et al., 1949). It is suggested

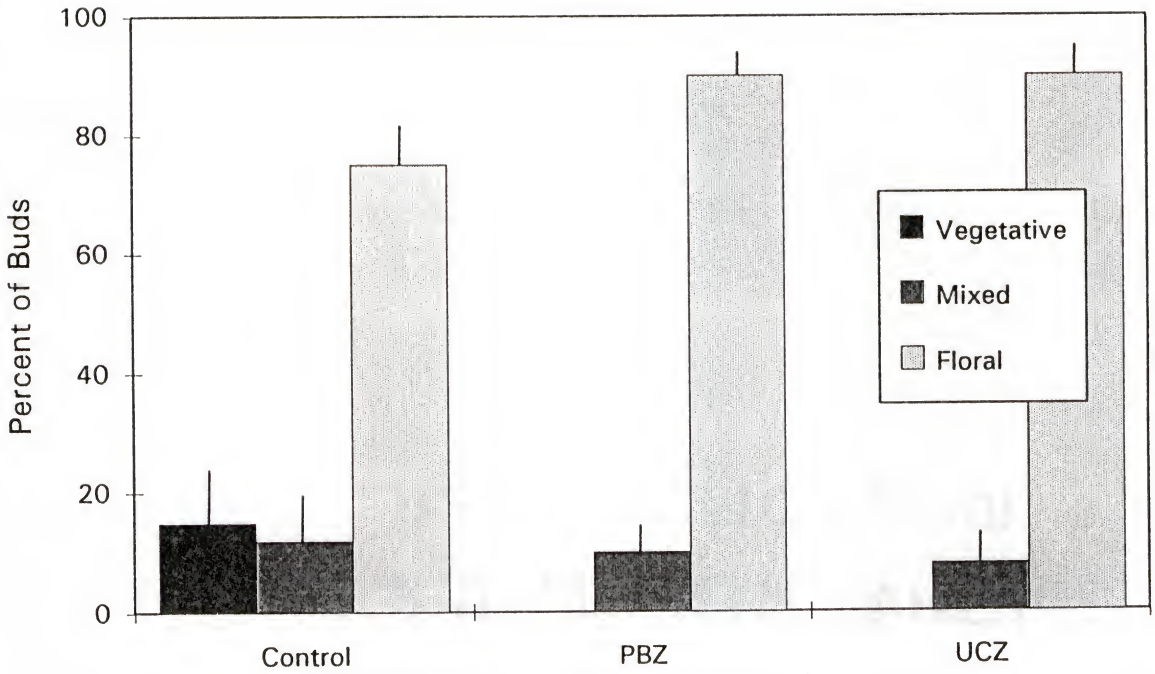

Figure 11-3. Apical bud morphogenesis in 'Tommy Atkins' mango in response to a spray of 2,000 $mg \cdot l^{-1}$ paclobutrazol (PBZ) or 500 $mg \cdot l^{-1}$ uniconazole (UCZ) and subsequent chilling treatment at 18/10°C. Vegetative buds produced shoot extension growth; floral buds formed floral panicles bearing only flowers; and mixed buds formed mixed panicles bearing leaves and axillary flower clusters.

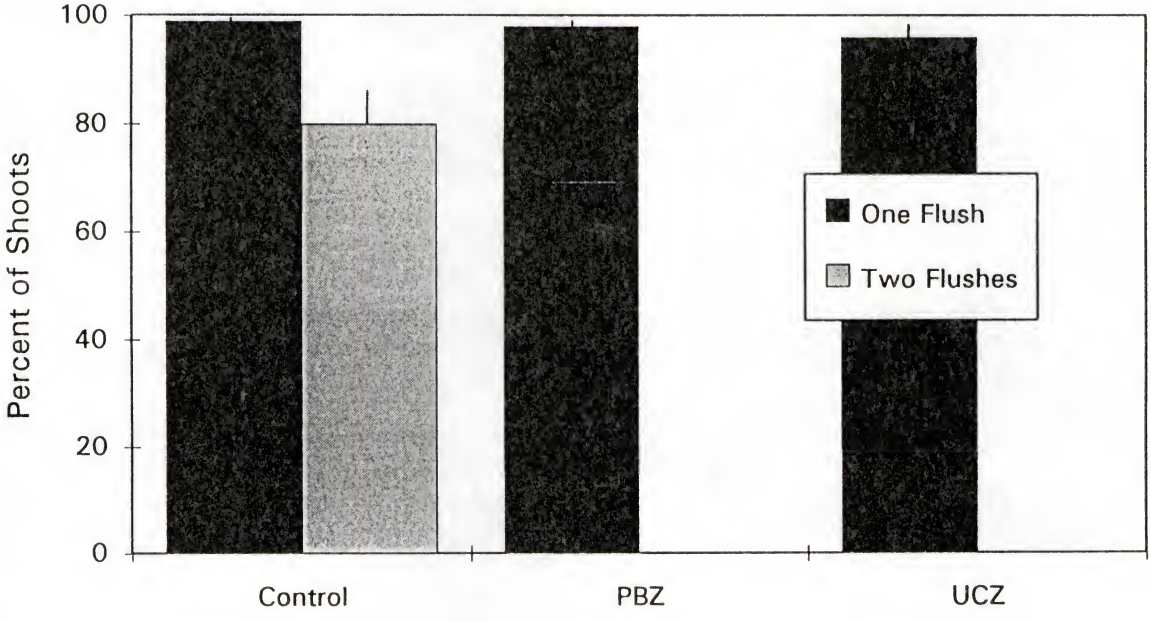

Figure 11-4. Vegetative flushing of 'Tommy Atkins' mango as affected by a spray of 2,000 mg.l-1 paclobutrazol or 500 mg.l-1 uniconazole and subsequent treatment at 30/25°C. During the 75 days of temperature treatment, shoots of control trees flushed twice, while shoots of triazole-treated trees flushed only once and had shorter internodes.

that floral buds (74%) differentiated entirely during chilling treatment, and thus expressed full reproductive morphogenesis. Mixed buds (11%) achieved only fractional induction perhaps due to suboptimal level or translocation of the floral stimulus, its lack of receptivity by meristematic tissues, or to partial vegetative differentiation prior to arrival of the stimulus. Vegetative buds (15%) are thought to have resulted from bud development in the absence of the floral stimulus, either by its delayed arrival or suboptimal production. In previous studies, chilling also induced flowering of several mango cultivars (Shu and Sheen, 1987; Whiley et al., 1989).

The results show that PBZ or UCZ did not cause floral induction because vegetative, instead of reproductive (mixed or floral) buds were formed at 30/25°C despite PBZ or UCZ pre-treatment. PBZ- and UCZ-sprayed trees did, however, produce nearly 20% more floral buds than nonsprayed trees (91 and 93% vs. 74%), and attained earlier bud break. PBZ and UCZ possibly increased flowering rates by preventing shoot elongation prior to chilling treatment, whereas they may have caused rapid development of reproductive buds by interfering with gibberellin metabolism.

Reduction in vegetative flushing and internode length by both PBZ and UCZ demonstrates that the compounds reached developing buds. PBZ and UCZ are reported to affect mango

vegetative growth by interfering with gibberellin biosynthesis (Kulkarni, 1988; Rukayah, 1990; Burondkar and Gunjate, 1991; Charnvichit and Tangumpai, 1991).

Since artificial chilling can induce flowering of mango, low temperatures are thought to induce flowering in the subtropics. Yet, mango trees in the tropics can initiate flowers at temperatures above 20°C, apparently in response to water deficit (Singh, 1960; Hallé et al., 1978; Chacko, 1986). Because mango displays such wide reproductive plasticity, more research is needed to elucidate the control of bud morphogenesis and the nature of the evasive floral stimulus.

CHAPTER 12
SUMMARY, CONCLUSIONS, AND FUTURE PROSPECTS

The Experimental System

This study was conducted to gain information on the regulation of flowering in mango as it relates to developmental and environmental factors, and applied bioregulators. An experimental system was developed using container-grown trees generated by air-layering. These trees were demonstrated to be nonjuvenile, capable of initiating inflorescences within as few as three months after being detached from mother trees in the field. Selective pruning of air-layers produced canopies that were uniform and phenologically synchronized, thus reducing developmental variability among experimental plants.

A cool temperature treatment of 18°C day/10°C applied in growth chambers at any time of the year consistently and predictably caused floral initiation of container-grown trees bearing mature (at least seven weeks old) foliage at the time of starting the cool temperature treatment. Exposure to the cool temperature treatment typically caused more than 85% of vegetative shoots to initiate inflorescence

development. Defoliation or removal of shoot tips eliminated paradormancy imposed by the apical bud or leaves on target buds. Floral budbreak occurred within five weeks of starting the cool-temperature treatment, reducing the time required to achieve floral budbreak by at least four weeks in comparison with intact trees.

Main Conclusions

Flowering of mango occurs mainly in apical buds of vegetative shoots that have previously undergone periodic extension growth. Apical buds (as well as axillary buds along the shoot stem) remain dormant and nondifferentiated between episodes of extension growth of the shoot apical meristem. Dormancy release involves the resumption of bud growth and the start of bud differentiation irrespective of whether vegetative or inflorescence development is initiated. Floral induction causes dormant, non-differentited buds to become committed to inflorescence morphogenesis.

A leaf-generated floral stimulus of unknown identity is required for floral induction and for initiation of inflorescence primordia. Girdling prevents translocation of the stimulus. The stimulus appeared to persist for about 5 days in shoot stems after leaves were removed.

Enhanced ethylene production was assayed for its involvement in the transition to flowering. No changes in

endogenous ethylene production were detected in buds, leaves, or whole trees in containers as part of the events preceding floral initiation.

Continuous cool temperatures (18°C day/10°C night) caused production of inflorescences in this study, whereas continuous warm temperatures (30°C day/25°C night) promoted vegetative morphogenesis. Leaves achieved competence to respond to cool temperatures when they reached about seven weeks of age. Presumably, leaves become capable of synthesizing the floral stimulus upon reaching this age. A minimum period of about three weeks of exposure to cool temperatures was required for floral induction. Bud growth during exposure to cool temperatures was required for initiation of inflorescence morphogenesis. This requirement suggests that the floral stimulus loses effectiveness as temperature increases.

Mango buds consist of an apical meristem and a series of preformed nodes. Temperature shifts (from cool to warm conditions or vice versa) at different stages of bud differentiation revealed that the preformed nodes and apical meristem respond independently to prevailing temperatures at the time of their activation, resulting in expression of vegetative-to-floral or floral-to-vegetative transitional phenotypes in a single flush of growth.

Photoperiod was not found to be involved in floral induction in this study. Short or long photoperiods (11 or 13 h), when accompanied by warm temperatures (30°C day/25°C night), did not result in floral initiation, whereas short, long, neutral or continuous photoperiods (11, 12, or 13 h, or 24 h with 12-h thermoperiods) accompanied by cool temperatures (18°C day/10°C night) resulted mainly in floral initiation. Therefore, cool temperatures rather than a short photoperiod caused floral induction, whereas warm temperatures rather than a long photoperiod inhibited flowering.

Water stress has been considered to induce the transition to flowering in warm temperatures that would normally promote vegetative growth in irrigated trees. It was found during this study that water stress applied during warm, summer months repressed vegetative extension of shoots, but did not induce flowering. Trees water-stressed during cool, autumn months did not produce more flowering than irrigated ones, but they displayed earlier floral budbreak. Thus, water stress did not cause floral induction, but accelerated the growth of buds that had already attained floral induction as a result of exposure to cool temperatures.

The floral-inhibitory mode of action of exogenous gibberellin and the floral-promotive mode of action of

gibberellin-biosynthesis inhibitors were investigated by subjecting treated trees to floral-promotive (temperatures below 18°C) and nonfloral-promotive (temperatures above 25°C) temperature regimes. Exogenous GA_3 (10 to 250 $mg.l^{-1}$) delayed initiation of inflorescence development, but did not cause buds to grow vegetatively, when bud differentiation occurred under cool temperature conditions. GA_3 did not affect vegetative flushing of shoots under warm temperature conditions. GA_3, therefore, does not appear to affect floral induction, but the growth of dormant buds that have already attained floral induction in response to cool temperatures. Decreasing temperature appeared to decrease the effectiveness of gibberellin, and to increase effectiveness of the floral stimulus.

The gibberellin-biosynthesis inhibitors, paclobutrazol and uniconazole, advanced flowering but did not cause floral induction in cool temperatures, since both nontreated and treated trees flowered in floral-promotive temperatures. Neither inhibitor produced flowering when bud differentiation occurred in warm temperatures, but both compounds delayed vegetative flushing and caused internode shortening.

Future Prospects

Bud morphogenesis in mango appears to be strongly modulated by endogenous/environmental conditions prevailing

at the time buds are released from dormancy and resume growth. Manipulation of the time of flowering in mango should, therefore, aim at identifying treatments effective in releasing bud dormancy at times when environmental and tree conditions are conducive to initiation and complete differentiation of inflorescence primordia. Treatment efficacy may depend on cultivar, as 'Keitt' trees appeared to be more sensitive to bioregulators than 'Tommy Atkins' trees in this study, and on specific environmental conditions, particularly temperature and water availability. Cultivars such as 'Julie' in the Caribbean basin can initiate flowering under mildly inductive environmental conditions (warm, rainy weather) which typically promote vegetative growth in 'Tommy Atkins'.

Results of this study indicate that optimum conditions for floral initiation of 'Tommy Atkins' and 'Keitt' mango included temperatures about 20°C or lower for at least three weeks (Chapters 6 and 7), and tree canopies with mostly mature, floral-inductive leaves capable of responding to cool temperatures (Chapters 5 and 6). However, the temperature threshold that permits initiation of floral morphogenesis is possibly modified by interactions with plant water status or other environmental and endogenous factors. Further research in this area is warranted.

Water stress showed potential in preventing vegetative flushing close to the flowering period, and advancing floral budbreak (Chapter 9). Judicious water stress may possibly promote flowering in dry areas with marginally-inductive temperatures by preventing untimely vegetative flushing of shoots, and may advance the flowering and harvest periods. Bioregulators capable of prolonging or releasing bud dormancy (Chapters 10 and 11) in a predictable, economic, and safe manner appear to offer a valuable tool for the control of flowering in mango.

The experimental approaches developed during this study may be applied to future investigations of physiological, genetic, and molecular aspects of flowering in mango. Future flowering studies should take into account not only the separate regulation of bud dormancy and morphogenesis, but also the presence of separate morphogenic regions within prospective flowering buds.

LITERATURE CITED

Andrews, L. and Le Fook, U. 1985. Effects of growth regulators on flowering pattern, flower suppression and fruit set in mango (*Mangifera indica* L. cv. Julie). Joint Proc. 21st Ann. Meet. Carib. Food Crops Soc. & 32nd Ann. Meet. Amer. Soc. Hortic. Sci. -Tropical Region. Port of Spain, Trinidad. pp. 61-65.

Anon. 1991. FAO 1990 Production Yearbook. Vol. 43, FAO, Rome.

Barba, R. C. 1974. Induction of flowering of the mango by chemical spray. Crop. Sci. Soc. Philipp. Proc. 5:154-160.

Basu, R. N. 1972. Rooting in cuttings and air-layers of mango. Acta Hortic. 24:61-64.

Beal, P. R., and Newman, G. A. 1986. Climatic influences on mango production in the Bowen district. Qsld. J. Agric. Anim. Sci. 43:83-89.

Bernier, G. 1988. The control of floral evocation and morphogenesis.Ann. Rev. Plant Physiol. and Plant Mol. Biol. 39:175-219.

Bernier, G., 1993. Physiological signals that induce flowering. The Plant Cell 5:1147-1155.

Bernier, G., Kinet, J. M., and Sachs, R. M. 1981. The Physiology of Flowering. Vol. 1. CRC Press, Boca Raton, FL.

Bondad, N. D., and Linsangan, E. 1979. Flowering in mango induced with potassium nitrate. HortScience 14:527-528.

Buban, T., and Faust, M. 1982. Flower bud induction in apple trees: internal control and differentiation. Hort. Rev. 4:174-203.

Bueno, P. B., and Valmayor, R. V. 1974. Key to mango flowering. Agr. Los Baños 13:4.

Burondkar, M. and Gunjate, R. T. 1991. Regulation of shoot growth and flowering habit in 'Alphonso' mango with paclobutrazol. Acta Hort. 291:79-84.

Chacko, E.K. 1986. Physiology of vegetatvie and reproductive growth in mango (*Mangifera indica* L.) trees. Proc. First Australian Mango Research Workshop. Cairns. pp 54-70.

Chacko, E. K. 1991. Mango flowering - still an enigma. Acta Hort. 291:12-21.

Chacko, E. K., and Ranhawa, G. S. 1971. Towards an understanding of the factors affecting flowering in mango. Andhra Agr. J. 18:226-236.

Chacko, E. K., Kohli, R. R., and Randhawa, G. S. 1972. Studies on the effect of 2-chloroethyl phosphonic acid (Ethrel) on mango. I. Flower induction in "off" year in Langra trees. Indian J. Hort. 29:1-4.

Chacko, E. K., Kohli, R. R., and Randhawa, G. S. 1974. Investigations on the use of 2-chloroethyl phosphonic acid (Ethephon, CEPA) for the control of biennial bearing in mango. Scientia Hort. 2:389-398.

Chacko, E. K., Kholi, R. R., Swamy, R. D., and Randhawa, G. S. 1974. Effect of 2 chloroethyl phosphonic acid on flower induction in juvenile mango (*Mangifera indica*) seedlings. Physiol. Plant. 32:188-190.

Chadha, K. L. and Pal, R. N. 1986. *Mangifera indica*. In: A.H. Halevy (Ed.) Handbook of Flowering. CRC Press, Boca Raton, Florida, Vol. V pp 211-230.

Charnvichit, S., Tongumpai, P., Sanguansupyakorn, C., Phavaphutanon, L. and Subhardrabandhu, S. 1991. Effect of paclobutrazol on canopy size control and flowering of mango. cv. Nam Dok Mai No. 4 after hard pruning. Acta Hortic. 291:60-66.

Chasan, R. and Walbot, V. 1993. Mechanism of plant reproduction: questions and approaches. The Plant Cell 5:1139-1146.

Chaudri, S. A. 1976. Mangifera indica - Mango. In: Garner, R. J. and S. A. Chaudri (Eds.). The Propagation of Tropical Fruit Trees. FAO, Commonwealth Agricultural Bureaux, England. pp. 403-474.

Chen, W. S. 1985. Flower induction in mango (*Mangifera indica* L) with plant growth substances. Proc. Natl. Sci. Counc. B. ROC. 9:9-12.

Chen, W. S. 1987. Endogenous growth substances in relation to shoot growth and flower bud development of mango. J. Amer. Soc. Hort. Sci. 112:360-363.

Choudhuri, J. M., and Rudra, P. 1971. Physiological studies on chemical control of growth and flowering in mango (*Mangifera indica* L.). Indian Agr. 15:127-135.

Christianson, M. L. 1985. An embryogenic culture of soybean: Towards a geneal theory of somatic embryogenesis. In: Henke, R. R., Hughes, K. W., Constantin, M. P. and Holleander, A. (Eds.). Tissue Culture in Forestry and Agriculture. Plenum, New York, pp. 83.103.

Christianson, M. L. and Warnick, D. A. 1983. Competence and determination in the process of in vitro shoot organogenesis. Dev. Biol. 95:288-293.

Cull, B. W. 1987. A whole plant approach to productivity research for mango. In: R. T. Prenseley and G. Tucker. (Eds.). Mango - a review. Commonwealth Science Council, London. pp. 19-28.

Cull, B. W. 1991. Mango crop management. Acta Hort. 291:154-173.

Davenport, T. L. 1983. Daminozide and gibberellin effects on floral induction of *Citrus latifolia*. HortScience 18:947-949.

Davenport, T. L. 1990. Citrus flowering. Hort. Rev. 12:349-408.

Davenport, T. L. 1993. Floral manipulation in mangos. In: Chia, C. L. and Evans, D. O. (Eds.). University of Hawaii at Manoa, pp. 54.60

Dutcher, R. D. 1972. Induction of early flowering in 'Carabao' mango in the Philippines by smudging and ethephon application. HortScience 7:343.

Fosket, D. E. 1994. Plant Growth and Development - A Molecular Approach. Academic Press, New York, 580 pp.

Galán-Saúco, V. 1990. Los Frutales Tropicales en los Subtrópicos. I. Aguacate, Mango, Litchi y Longan. Ediciones Mundi-Prensa. Madrid. pp. 133.

Galán Saúco, V., Fernandez-Galvan, D., Hernandez-Delgado, P. and Garcia, P. 1993. Comparison of manual and ethephon-induced deblossoming of mango cv. Keitt in the Canary Islands. Acta Hortic., 361:248-255.

Goguey, T. 1990. Effets d'applications répétées de CULTAR (Paclobutrazol) sur *Mangifera indica* L. var. Valencia. Fruits 45:599-607.

Gonzales, L. G. 1923. The smudging of mango trees and its effects. Philipp. Agr. 12:15-27.

Goldschmidt, E. E. and Monselise, S. P. 1972. Hormonal control of flowering in *Citrus* and other woody perennials. p. 758-766 In: D. J. Carr (ed.) Plant Growth Substances, Springer Verlag, New York.

Grierson, W., Soule, J., and Kawada, K. 1982. Beneficial aspects of physiological stress. Hort Rev 4:247-271.

Guardiola, J. L. 1981. Flower initiation and development in *Citrus*. Proc. Int. Soc. Citriculture 1:242-248.

Guardiola, J. L., Agustí, M. and Garcia-Marí, F. 1977. Gibberellic acid and flower bud development in sweet orange. Proc. Int. Soc. Citriculture 2:696-699.

Guardiola, J. L., Monerri, C. and Agusti, M. 1982. The inhibitory effect of gibberellic acid on flowering in *Citrus*. Physiol. Plant. 55:136-142.

Hallé, F., Oldeman, R. A. and Tomlinson, P. B. 1978. Tropical Trees and Forests: an Architectural Analysis. Springer Verlag, Berlin, 441 p.

Hillier, G. R. 1991. Promotion of regular fruit cropping in mango with Cultar. Acta Hortic. 291:51-59.

Issarakraisila, M. and Considine, J. A. 1991. Pattern of vegetative and reproductive growth of mango trees in a warm temperate region of western Australia. Acta Hortic. 291:188-197.

Jackson, D. I. and G. B. Sweet. 1972. Flower initiation in woody temperate plants. Hort. Abstr. 42:9-24.

Kachru, R. B., Singh, R. N. and E. K. Chacko. 1971. Inhibition of flowering in mango (*Mangifera indica* L.) by gibberellic acid. HortScience 6:140-141.

Kinet, J. M. 1993. Environmental, chemical, and genetic control of flowering. Hort. Rev. 15:279-334.

Kozlowski, T. T., Kramer, P. J. and Pallardy, S. G. 1991. The physiological ecology of woody plants. Academic Press, New York, 657 pp.

Kulkarni, V. J. 1986. Graft-induced off-season flowering and fruiting in the mango. J. Hort. Sci. 61:141-145.

Kulkarni, V. J. 1988a. Chemical control of tree vigour and the promotion of flowering and fruiting in mango (*Mangifera indica* L.) using paclobutrazol. J. Hort. Sci. 63:557-566.

Kulkarni, V. J. 1988b. Further studies on graft-induced off-season flowering in mango (*Mangifera indica* L.). J. Hortic. Sci. 63:361-367.

Kulkarni, V. J. 1991. Physiology of flowering in mango studied by grafting. Acta Hortic. 291:95-104.

Lang, G. A., Early, J. D., Martin, G. C. and Darnell, R. L. 1987. Endo-, para-, and ecodormancy: physiological terminology and classification for dormancy research. HortScience 22:371-377.

Lanuza, E. A. 1937. Notes on bud differentiation in mango (*Mangifera indica* L.). Philipp. J. Agric. 10:131-151.

Larson, K. D., Schaffer, B. and Davies, F. S. 1989. Effect of irrigation on leaf water potential, yield and growth of mango trees. Proc. Fla. State Hort. Soc. 102:226-228.

Lord, E., and Bernier, G. 1989. Plant Reproduction, from Floral Induction to Pollination. Amer. Soc. Plant Physiol. Symp. Series, Vol. I.

Lord, E. M. and Eckard, K. J. 1987. Shoot development in *Citrus sinensis* L. (Washington Navel orange). II. Effects of GA_3 on flowering shoots. Bot. Gaz. 148:17-22.

Maity, S. C., Maity, S. K., and Sen, P. K. 1972. Effect of ethylene producing chemicals on growth and flowering of mango (*Mangifera indica* L.). Indian Agr. 16:201-203.

McDaniel, C. N. 1989. Floral initiation as a developmental process. In: Lord, E., and Bernier, G. (Eds.) Plant Reproduction, from Floral Induction to Pollination. Amer. Soc. Plant Physiol. Symp. Series, Vol. I., pp. 51-57.

McDaniel, C. N., Singer, S. R., Gebhardt, J. S. and Dennin, K. A. 1987. Floral determination: A critical process in meristem ontogeny. In: The Manipulation of Flowering, J. G. Atherton (ed.). Butterworth, London., pp. 109-120.

McDaniel, C. N., Singer, S. R. and Smith, S. M. E. 1992. Developmental states associated with the floral transition. Dev. Biol. 153:59-69.

Menzel, C. M., Rasmussen, T. S. and Simpson, D. R. 1989. Effects of temperature and leaf water stress on growth and flowering of litchi (*Litchi chinensis* Sonn.). J. Hort. Sci. 64:739-752.

Menzel, C. M., and Simpson, D. R. 1990. Effect of environment on growth and flowering of lychee (*Litchi chinensis* Sonn.). Acta Hortic. 275:161-166.

Mosqueda-Vázquez, R., and Avila-Reséndiz. C. 1985. Inducción floral del mango con aplicaciones de KNO_3 y su inhibición al aplicar $AgNO_3$ o $CoCl_2$. Horticultura Mexicana 1:93-101.

Mosqueda-Vázquez, R., and de los Santos de la Rosa, F., 1981. Aspersiones de nitrato de potasio para adelantar e inducir la floración del mango cv. Manila en México. Proc. of the Tropical Region Amer. Soc. Hort. Sci. 25:311-316.

Moss, G. I., 1976. Temperature effects on flower initiation in sweet orange (*Citrus sinensis*). Aust. J. Agric. Res. 21:233-242.

Mustard, M. J. and Lynch, S. J. 1946. Flower-bud formation and development in *Mangifera indica* L. Bot. Gaz. 108:136-140.

Nakasone, H. Y., Bowers, F. A. I. and Beaumont, J. H. 1955. Terminal growth and flowering behavior of the Pirie mango (*Mangifera indica* L.) in Hawaii. Proc. Amer. Soc. Hort. Sci. 66:183-191.

Núñez-Elisea, R. 1985. Flowering and fruit set of a monoembryonic and polyembryonic mango as influenced by potassium nitrate sprays and shoot decapitation. Proc. Florida State Hort. Soc. 98:179-183.

Núñez-Elisea, R. 1986. Producción temprana de mango 'Haden' y 'Manila' con aspersiones de nitrato de potasio. SARH-INIFAP-CIAPAC, Campo Agricola Experimental Tecomán. Colima, México, Folleto para productores No. 8.

Núñez-Elisea, R. 1988. Nitrato de amonio: nueva alternativa para adelantar la floración y cosecha del mango. SARH-INIFAP-CIFAP COLIMA, Campo Experimental Tecomán. Colima, México. Desplegable para productores No. 4.

Núñez-Elisea, R., and Davenport, T. L. 1983. Abscission and ethylene production of mango (*Mangifera indica* L.) fruit cv. Tommy Atkins. Proc. Florida State Hort. Soc. 96:185-188.

Núñez-Elisea, R., and Davenport, T. L. 1986. Abscission of mango fruitlets as influenced by enhanced ethylene biosynthesis. Plant Physiol. 82:991-994.

Núñez-Elisea, R., and Caldeira, M. L. 1988. Induction of flowering in mango (*Mangifera indica* L.) with ammonium nitrate sprays. HortScience 23:833.

Núñez-Elisea, R., Becerril-Roman, A. E., and Diaz-Montenegro, D. 1979. Efectos del ethrel sobre la floración en mango cv. Haden. Proc. Trop. Region Amer. Soc. Hort. Sci. 23:138-140.

Núñez-Elisea, R., Caldeira, M. L. and Davenport, T. L. 1990. Thidiazuron effects on growth initiation and expression in mango. (Abstr.) HortScience 25:1167-1168.

Ou, S. K. 1982. Temperature effect on differential shoot development of mango during flowering period. J. Agric. Res. China 31:209-212.

Pal, R. N. and Chadha, K. L. 1982. Deblossoming mangoes with cycloheximide. J. Hortic. Sci. 57:331-332.

Pandey, R. M., Singh, R. N., and Sinha, G. C. 1973. Usefulness of ethrel in regulating flower bearing in mango. Sci. Cult. 39:148-150.

Pharis, R. P. and King, R. W. 1985. Gibberellins and reproductive development in seed plants. Ann. Rev. Plant Physiol. 36:517-568.

Rao, V. N. M. 1967. Propagation practices. In: Kurup, C. G. R. (Editor). The mango handbook. Indian Council of Agricultural Research, New Delhi, pp. 32-69.

Ravishankar, H., Rao, M. M. and Bojappa K. M. 1979. Fruit-bud differentiation in mango 'Alphonso' and 'Totapuri' under mild tropical rainy conditions. Scientia Hortic. 10:95-99.

Rawash, M. A., Hamady, A. E. and Nabawy, S. E. 1983. Regulation of flowering and fruiting in mango trees by using some growth regulators. Ann. Agric. Sci. Univ. of Ain Shams (Egypt) 28:227-240.

Reece, P. C., Furr, J. R. and Cooper, W.C. 1946. The inhibiting effect of the terminal bud on flower formation in the axillary buds of the 'Haden' mango. Amer. J. Bot. 33:209-210.

Reece, P. C., J. R. Furr and C. W. Cooper. 1949. Further studies of floral induction in the 'Haden' mango (*Mangifera indica* L.). Amer. J. Bot. 36:734-740.

Rowley, A. J. 1990. The effect of Cultar applied as a soil drench on 'Zill' mango trees. Acta Hortic. 275:211-215.

Rukayah, A. 1990. The effects of uniconazole on flowering and fruiting of mango clone "MA 125". Abstracts of contributed papers. XXIII Intnal. Hort. Congress, Firenze, Italy. Abstr. No. 4255.

Saidha, T., Madhava Rao, V. N., and Santhanakrishnan, P. 1983. Internal leaf ethylene levels in relation to flowering in mango. Indian J. Hort. 40:139-146.

Schaffer, B., Whiley, A. W. and Crane, J. H. 1994. Mango. In: Schaffer, B. and Andersen, P. C. (Eds.) Handbook of Environmental Physiology of Fruit Crops. Vol II. Subtropical and Tropical Crops. CRC Press, Inc. Boca Raton, Florida, pp 310.

Scholander, P. F., Hammel, H. T., Bradstreet, E. D., and Hemmingsen, E. A. 1965. Sap pressure in vascular plants. Science 48: 339-345.

Scholefield, P. B., Oag, D. R. and Sedgley, M. 1986., The relationship between vegetative and reproductive development in the mango in northern Australia. Aust. J. Agric. Res. 37:425-433.

Schwabe, W. W. 1987. The flowering problem. In: J. G. Atherton (Ed.) Manipulation of flowering. Butterworths, London, pp 3-13.Sedgley, M., 1990. Flowering of deciduous perennial fruit crops. Hort. Rev. 12:223-264.

Sen, P. K. and Mallik, P. C. 1941. The differentiation of the flower bud of the mango. Ind. Jour Agr. Sci. II (Part I):74-79.

Sen, P. K., Bandopadhyay, M., Roy, S. S., and Basu, R.N. 1973. Use of ethrel in controlling non-uniform bearing of mango (*Mangifera indica* L.) Indian Agr. 17:285-288.Shu, Z. H. and Sheen, T. F., 1987. Floral induction in axillary buds of mango (*Mangifera indica* L.) as affected by temperature. Scientia Hortic. 31:81-87.

Singh, L. B. 1954. Propagation of mango by air-layering for rootstocks. Proc. Amer. Soc. Hort. Sci. 63: 128-130.

Singh, L. B. 1959. Movement of flowering substances in the mango (*Mangifera indica* L.) leaves. Hort. Adv. 3:20-27.

Singh, L. B. 1960. The mango- botany, cultivation, and utilization. Leonard Hill, London.

Singh, R. N. 1958. Studies in the differentiation and development of fruit buds in mango. II. Morphological and histological changes. Hort. Adv. 3:37-43.

Singh, R. N. 1978. Mango. Indian Council of Agricultural Research. New Delhi. 99 pp.

Singh, S. N. and Teaotia, S. S. 1951. Effect of some hormones on the rootage of mango. Sci. and Cult. 17:207-210.

Southwick, S. M., and Davenport, T. L. 1986. Characterization of water stress and low temperature effects on flower induction in *Citrus*. Plant Physiol. 81:26-29.

Steeves, T. A. and Sussex, I. M. 1989. Patterns in Plant Development, 2nd ed. Cambridge: Cambridge University Press.

Srivastava, R. P. 1960. Studies in the responses of plant growth regulators in air-layered shoots of mango (*Mangifera indica* L.). J. Sci. Res. Banaras Hindu Univ. 11:1-3.

Suryanarayana, V. and Rao, V. N. M. 1978. Effect of growth retardants on certain biochemical changes in relation to flowering. Indian J. Plant Physiol. 21:1-6.

Thomas, B. and Vince-Prue, D. 1984. Juvenility, photoperiodism and vernalization. In: Wilkins, M. B. (Ed.) Advanced Plant Physiology. Pitman, London, 514 pp

Thuck-thye L. 1978. Ethylene and the induction of flowering by potassium nitrate in mango (*Mangifera indica* L.). M. Sc. Thesis. University of the Philippines at los Baños. The Philippines.

Tomer, E. 1984. Inhibition of flowering in mango by gibberelic acid. Scientia Hortic. 24:299-303.

Tongumpai, P., Jutamanee, K. and Subhadrabandhu, S. 1991. Effect of paclobutrazol on flowering of mango cv. Khiew Sawoey. Acta Hortic. 291:67-70.

Valmayor, R. V. 1968. The mango. Its botany and production. University of the Philippines, College of Agriculture, Laguna, Philippines. 90 pp.

van der Meulen, J. H. E., Smith, T., van den Boom, I. B., Kok, A, Schwartz, C. and Jacobs, J. 1971. Mango growing in South Africa. Citrus and Subtropical Fruit Research Institute, Nelspruit. Leaflet No. 48. 40 p.

Verheij, E. W. M. 1986. Towards a classification of tropical fruit trees. Acta Hortic. 175:137-149.

Voon, C. H., C. Pitarpaivan and C. Pei. 1991. Mango cropping manipulation with Cultar. Acta Hort. 291:219-228.

Wang S. Y., Steffens G. L., and Faust M. 1986. Breaking bud dormancy in apple with a plant bioregulator, thidiazuron. Phytochemistry 25:311-317.

Whiley, A. W. 1993. Environmental effects on phenology and physiology of mango- a review. Acta Hortic. 341:168-176.

Whiley, A. W., Rasmusen, T. S., Saranah, J. B. and Wolstenholme, B. N. 1989. Effect of temperature on growth, dry matter production and starch accumulation in ten mango (*Mangifera indica* L.) cultivars. J. Hort. Sci. 64:753-765.

Whiley, A. W., T. S. Rasmusen, B. N. Wolstenholme, J. B. Saranah and B. W. Cull. 1991. Interpretation of growth responses of some mango cultivars grown under controlled temperatures. Acta Hort. 291:22-31.

Wolstenholme, B. N. and Hofmeyr, D. 1985. Effects of various floral induction treatments on container-grown mango trees. S. Afr. Mango Grow. Assoc. Res. Rep. 5:36-38.

Wolstenholme, B. N. and Mullins, P. C. 1982. Flowering, pollination and fruit set of mango- a discussion of limiting factors. Res. Rep. South Afican Mango Growers Assoc. 2:5-11.

Young, T. W. and Sauls, J. W. 1981. The mango industry in Florida. Fla. Coop. Exp. Serv. Bull. 189. Univ. of Florida, 70 p.

Zeevart, J. A. D. 1976. Physiology of flower formation. Ann. Rev. Plant. Physiol. 27:321-348.

BIOGRAPHICAL SKETCH

Roberto Núñez-Elisea was born on December 11, 1956, in Manzanillo, Colima, Mexico. He received a bachelor's degree in agronomy/horticultural science at the Universidad Autónoma Chapingo, in Mexico, in 1978. The author worked in research on tropical fruit production at the Instituto Nacional de Investigaciones Agrícolas in Jalisco, Mexico, from 1979 to 1981. He attended the University of Florida from 1981 to 1983 and was awarded a Master of Science degree in horticultural science (fruit crops). From 1984 to 1988, the author conducted research on mango crop regulation at the Instituto Nacional de Investigaciones Forestales y Agropecuarias in Colima, Mexico. In 1988 he started a doctoral program at the University of Florida, and is currently a candidate for the degree of Doctor of Philosophy in horticultural science.

The author is married to Maria Lilia Caldeira and has an eight-year-old daughter, Ilse del Carmen Núñez Zuazo, and a three-year-old son, Roberto Núñez.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Thomas L. Davenport, Chair
Associate Professor of Horticultural Science

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Bruce Schaffer, Cochair
Professor of Horticulutral Science

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Rebecca L. Darnell
Associate Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

J. Thomas Mullins
Professor of Botany

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Richard C. Smith
Professor of Botany

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1994

Jack L. Fry

Dean, College of Agriculture

Dean, Graduate School